Observations on the breeding biology of *Ambassis agassizii* Steindachner, 1867 (Teleostei: Ambassidae) from the Murray Darling Basin in New South Wales

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ABSTRACT

Ambassis agassizii (Agassiz's Glassfish) collected from Barren Box Swamp was bred in ponds at the Narrandera Fisheries Centre on ten occasions between 1968 and 1971 when surface water temperatures were 19.0 - 27.0°C, between November and early January, and again in February. Breeding conditions and limited embryological development were determined. A single, demersal, transparent, spherical, telolecithal and slightly adhesive egg was collected on 9.xii.70 at 23.0°C. It possessed a single oil globule; and measured 0.61mm in diameter. Eggs hatched in about a day at temperatures between 20.0 and 29.0°C. The smallest larva collected was 2.59mm, and was about 1.6mm at hatching. The pro-larval stage terminated at under 10 days after hatching. The largest male and female adults were 60.0 and 68.0mm (TL) and 2.51 and 3.88g respectively. Egg counts were 380 to 9966 (mean 3257) with higher counts in larger fish. Ovaries generally had two size ranges of oocytes. Spawning was multiple. The GSI rose to 10.50 and 8.86 in females and males respectively. The breeding of *A. agassizii* was compared with the coastal population and some other ambassids, and some comparisons are made with other inland NSW species.

Key words: *Ambassis agassizii*, Agassiz's Glassfish, Chanda Perch, breeding biology, development in ambassids, status.

Introduction

Breeding experiments on Murray Darling freshwater fish, particularly small forage species, were carried out between 1964 and 1973 at the Narrandera Fisheries Centre (then the Inland Fisheries Research Station) to assist with identification of eggs and larvae, and to attain a better understanding of the environmental conditions necessary for effective breeding and recruitment (Llewellyn 1971, 1973, 1974, 1979, 2005) so as to assist with their management. At the time, government research was directed towards increasing and maintaining stocks of fishes of commercial and recreational value, and forage fish were seen as an important part of the food chain for the large fishes of economic importance.

The Murray Darling Agassiz's Glassfish, *Ambassis agassizii* Steindachner 1867, is a little-studied inland fish, for which a better understanding of the environmental cues and factors necessary for breeding is crucial to its management and survival. The Family Ambassidae, commonly called Glassfishes, contains around 41 species worldwide, in 8 genera, of which around 13 species are known from Australia (Allen and Burgess 1990). Agassiz's Glassfish (also known as the Western Chanda Perch, Silver Spray or Doody), previously known as *Ambassis castelnaui* (Macleay 1881), is the only species known to occur in the warmer, inland freshwaters of the Murray Darling Basin west of Narrandera. *Ambassis nigripinnis* occurs on the coast between Lake Hiawatha, NSW and the Mowbray River north of Cairns, Queensland (Allen *et al.* 2002), and it has been synonymised with *A. agassizii* by Allen and Burgess (1990). These two populations, the coastal population and the Murray Darling population, occur in two very different riverine environments with very different flow regimes. Most of the Ambassidae are estuarine but the Murray Darling population of *A. agassizii* is restricted to freshwater.

Some reproductive biology has been described for some species of Australian ambassids (*Ambassis macleayi* Semple 1985a; *Ambassis agrammus* Semple 1985b; both these species Bishop *et al.* 2001), and for coastal populations of *A. agassizii* (previously known as *A. nigrippinis* Milton and Arthington 1985). However very little information is available for the inland population of *A. agassizii* in the Murray Darling Basin (Koehn and O'Connor 1990), other than the brief comment on the breeding of *A. castelnaui* (now *A. agassizii*) in Llewellyn (1980, 1983). No new information has been published on the Murray Darling population of this species in the 30 years since this work was undertaken.

This study investigates the breeding and development of *A. agassizii* from the Murray Darling population, and compares it with that of the coastal population and with some other species in the family Ambassidae. The eggs and spawning of *A. agassizii* are compared also with other fish species occurring in the Murray Darling waters of New South Wales. The distribution and status of the Murray Darling population of *A. agassizii* is discussed based on records and a survey at that time.

Methods

Collection and sampling

The methods by which *A. agassizii* was sampled were essentially similar to that described in Llewellyn (2005), except bait traps were not successful. Again, because of the difficulty and unpredictability of collecting this species, all collecting was opportunistic during other sampling programs. When fish were caught, a major emphasis was placed on acquiring live fish for breeding experiments.

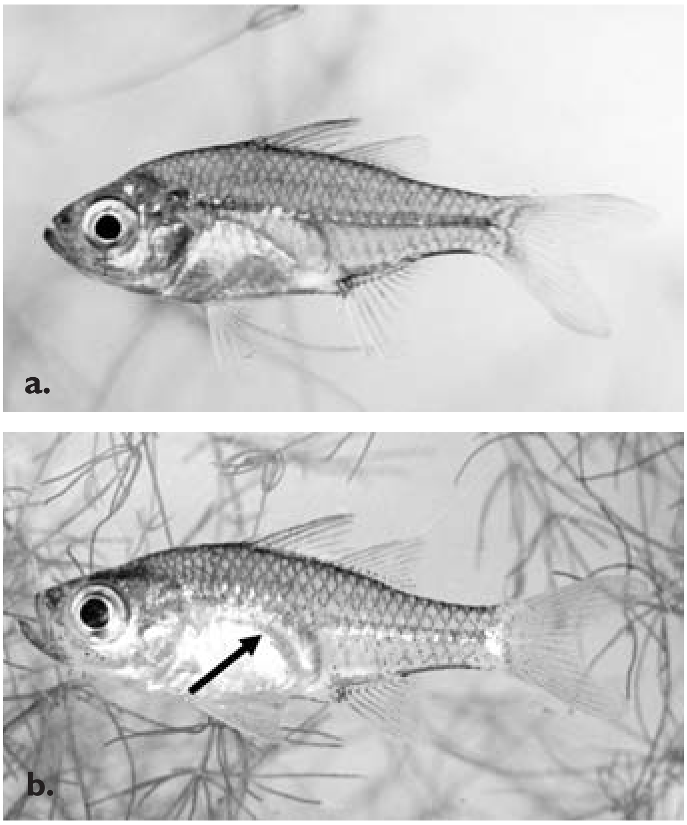


Figure 1. Adult *A. agassizii* from Willow Dam, a) Male and b) female. Note the line along the dorsal edge of the abdomen marked with an arrow, more rounded in the female.

A. agassizii (Fig. 1) was caught occasionally in the Murray Darling drainage west and north of the Narrandera Fisheries Centre. The site where it was caught most often was 23km west-north-west of Griffith, at Willow Dam, Barren Box Swamp, (34°11'S and 145°50'E), a site which was sampled frequently, but irregularly. A description of the site, methods of sampling at this site, and transportation of fish are described in Llewellyn (1974, 2005). The sampling methods used were efficient and effective, allowing for most fish within the vicinity of the weirs to be caught. The numbers collected at this site (Table 1) facilitated breeding experiments in ponds and aquaria at the Research Station. The only other sites where this species was recorded during 1960-1975 were Murray River at Wentworth, Bokhara River between Enngonia and Brewarrina, Narran River, Macintyre River, Lake Brewster and Lake Cargelligo (Table 1).

Determination of time of breeding

All dead fish were examined, dissected and the state of their gonads determined. The few fish, which died during collection and transport, together with some caught from ponds as the breeding season approached, were used for this purpose. These data facilitated assessment of the approximate time of the year of the breeding period and the actual onset of breeding. If gonads were of sufficient size and were easily recognisable, they were weighed and measured and a Gonosomatic index (GSI) was determined (GSI=(Weight of gonadx100)/Weight of body (Mackay 1973, Belsare 1962)). Counts of the number of ova present in ovaries were carried out wherever possible.

In addition, all dead fish and live fish when handled, where possible, were measured (total length TL and caudal fork CF) and weighed, and the time of sampling recorded. Total length measurements were used to create length frequency plots to ascertain the recruitment period, and to determine the annual growth rate. Selected external characters were also recorded in an attempt to determine the sex of specimens.

Breeding

All live fish collected were placed in small ponds around 0.01ha in area or in 90L aquaria.

Pond trials

Manipulation of ponds; records of stocking and de-stocking; egg, larvae and adult fish sampling from ponds; and temperature recording were carried out as reported by Llewellyn (2005). When gonad development of adults indicated that spawning may be imminent, regular sampling in the ponds using plankton nets and a small bottom scoop net were commenced to try to collect eggs and or larvae. The temperature records were used as in *G. rostratus* (Llewellyn 2005) to determine the temperature at which breeding occurred.

Aquarium trials

Some fish were placed in 90L aquaria planted with aquatic plants, particularly *Nitella* sp., and were fed plankton and other small organisms in an attempt to induce spawning, so that spawning could be scrutinized closely. Between 3 and 29 fish were placed in each aquarium and the periodicity of feeding was increased as the anticipated spawning period approached. An attempt was made to separate the sexes of some fish and then place them together when temperatures were warming up, in order to encourage breeding.

Development

Ova and larvae were handled as described in Llewellyn (2005) to determine their development; and drawings and measurements of these were made at various stages where possible. Using this information a description and timing of embryological development of eggs and larvae were pieced together. Temperature was recorded at intervals.

Results

Sampling strategy and times

The only area in which sufficient numbers of *A. ambassis* were collected to facilitate breeding trials was Willow Dam, Barren Box Swamp (Table 1). Fish were successfully caught using a beam push net in the ports of the weirs and a small haul net and dip net downstream, particularly after low flows had operated over the weir for a couple of days (Llewellyn 1974). One thousand and thirty three fish were collected at this site over six years from 1965 to 1970 on around 37 visits.

Table 1. Collections of *A. agassizii* 1960 to 1975. Comments include collector when not author, introduced species caught at same time when noted, gonad state of *A agassizii*, and FWFS, Freshwater Fish Survey of New South Wales (19,900 specimens in total) (Llewellyn 1983). *Collecting method at Willow Dam described in Llewellyn (1974).

Date	Location	Number	Temperature °C	Comment
14.iii.65	Willow Dam*	0	-	-
23.iii.65	Willow Dam	0	-	-
14.iii.66	Willow Dam	20	-	-
23.iii.66	Willow Dam	30	-	Gambusia and other species
18.x.66	Willow Dam	1	19.4	-
25.x.66	Willow Dam	0	21.0	-
2.xi.66	Willow Dam	0	-	-
7.xii.66	Willow Dam	0	-	-
19.i.67	Willow Dam	6	-	-
28.ii.67	Willow Dam	0	-	-
7.iii.67	Willow Dam	170	21.7	-
3.v.67	Willow Dam	0	-	-
25.v.67	Willow Dam	0	9.3-9.7	-
5.vi.67	Willow Dam	163	-	-
27.vii.67	Willow Dam	1	6.9-12.8	-
4.ix.67	Willow Dam	0	6.9-13.0	-
18.ix.67	Willow Dam	0	14.7-15.0	-
24.ix.67	Willow Dam	0	15.6	-
17.x.67	Willow Dam	4	-	-
2.xi.67	Willow Dam	129	21.7-24.4	Fish gravid. Redfin abundant
17.xi.67	Willow Dam	1	-	-
23.xi.67	Willow Dam	145	20.6-22.8	550+ Redfin
2.xii.67	Willow Dam	0	-	-
12.xii.67	Willow Dam	15	21.2-24.0	Sex organs not visible in some. 103 Redfin
26.xii.67	Willow Dam	0	-	-
17.i.68	Willow Dam	33	22.0-22.8	Thousands of Redfin,
14.ii.68	Willow Dam	4	23.9-26.3	Caught after flow commenced over boards. Gambusia and Redfin abundant.
19.ii.68	Willow Dam	4	-	Giving milt
20.iv.68	Willow Dam	0	-	-
4.vi.68	Willow Dam	0	-	-
7.vi.68	Willow Dam	0	-	Water flowing
20.vi.68	Willow Dam	0	-	Redfin absent
30.vi.68	Willow Dam	0	-	-
30.x.68	Willow Dam	0	-	-
22.v.69	Willow Dam	0	-	-
17.ii.70	Willow Dam	303	25.0	-
15.xii.70	Willow Dam	4+	25.5	-
27.x.72	Lake Brewster	2	-	Caught by beam trawl N. Ruello
1972-74	Lake Brewster - Lake Cargelligo	+	-	Miller (1976) in stomach contents of cormorants.
20.i.60	Bokhara River, between Brewarrina and Enngonia	2	-	Ripe. Together with numerous other species. N. Millward
1960	Narran Lake	-		-
Pre 1964	Murray River Wentworth	4	-	Collector ?
12.iv.75	Little Oxley R. MacIntyre R.	1		Rotenone. FWFS
7.x.75	Narran R. Angledool	1		Seine. FWFS
-	Unknown	6	-	+ 1 Sunfish
	Unknown	20	-	+ Smelt

The total and mean numbers of *A. agassizii* caught per visit for each month during this period is shown in Fig. 2. Most were caught during the summer period November to March, although one large catch (163) was taken on 5.vi.67 during winter (Table 1).

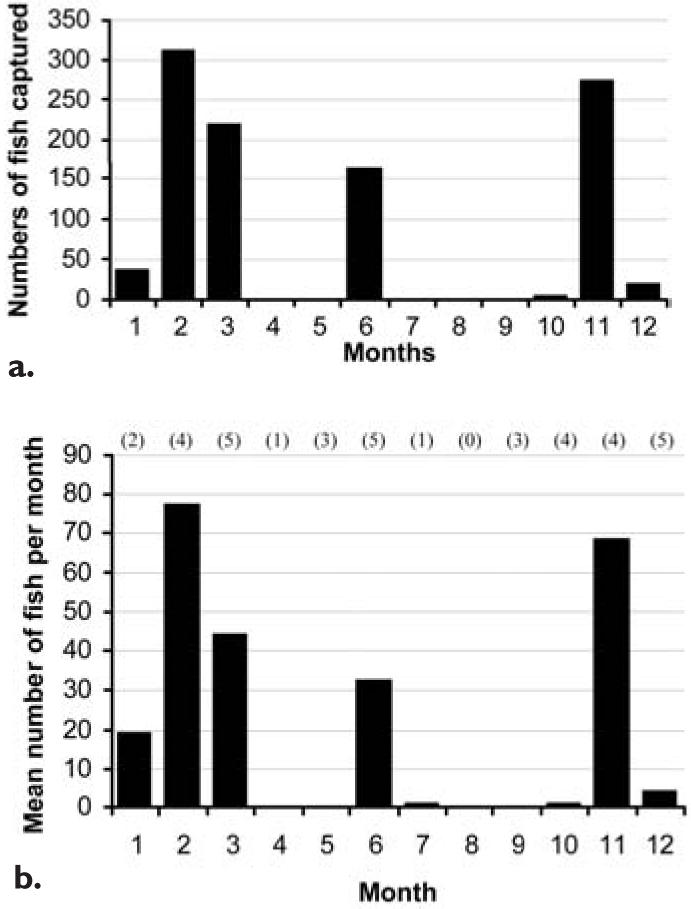


Figure 2 (a). Total numbers of *A. agassizii* caught in each month January to December (1-12), at Willow Dam between March 1965 and December 1970.

(b). Mean numbers of *A. agassizii* caught per visit in each month, at Willow Dam, between March 1965 and December 1970. Number of visits indicated in brackets.

Aquarium trials

Observations were made over numerous trials using both fish transferred from ponds, and fish collected from the wild. Fish were not particularly easy to keep in aquaria, and died off slowly over a period of several months. They were usually maintained in aquaria over the spring and summer period to attempt to induce spawning and were fed on plankton, small shrimp and other small invertebrates. Floating weeds were made available, because some other species were believed to spawn among floating weeds.

As the breeding season approached, the number of feeds per day was increased, and when breeding trials were carried out in cooler months, aquarium heaters were used to maintain a steady increase in temperature between 10 and 27°C. pH varied from 7.7 to 8.5.

Difficulty was experienced in sexing adult fish, although a difference existed in the shape of the internal line along the dorsal edge of the abdominal cavity where the dorsal edge of the gonads meets the abdominal wall (ie swim bladder) (Fig. 1a and b, 3a). This line is rounded in females and straight in males, and was more noticeable as breeding approached.

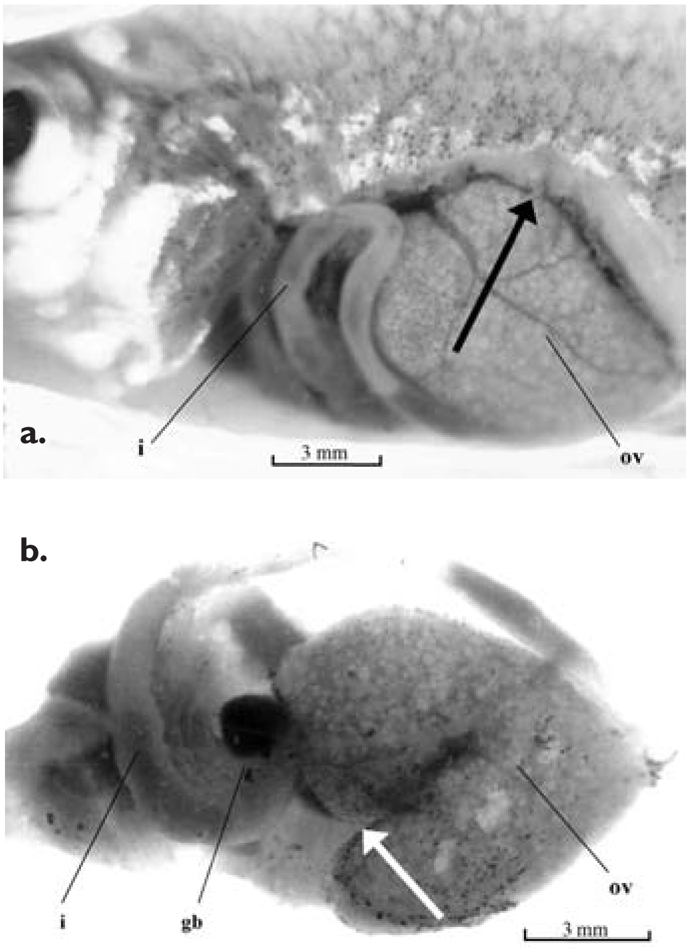


Figure 3. a) Window cut in abdomen to show the ovary and the noticeable curvature along the dorsal edge of ovary (black arrow) seen also in whole fish and typical of ripe females.

b) Ovary removed showing the uneven lobes and its bifid nature (white arrow). **ov**, ovary; **i**, intestine; **gb** gall bladder.

An attempt was made to breed two females and one male fish during winter. Fish were initially kept in separate 18L containers, fed copious plankton and water temperatures were simultaneously raised to 23.3°C over three weeks. The separated fish were easily agitated and did not put on much condition. However when they were put together in a 90L aquarium planted with water plants and maintained at 24.0°C, the male showed considerable interest in the two females, swimming up to each in turn and then around them and eventually chased them into the weeds. Water temperature in the tank was then raised slowly to 26.5°C, however no successful breeding was observed.

On 24.ii.67, when ambient aquarium water temperature had risen to 28.1°C, some fish did become quite ripe and spent time darting amongst the floating vegetation. One of these fish was stripped of some ova but the ova could not be fertilised. No successful breeding took place in any aquarium experiments.

Pond trials

Survival of *A. ambassis* stocked in ponds was variable. Losses were attributable to predation by cormorants and herons as well as low winter water temperatures. They were bred in ponds on at least 10 occasions between 1968 and 1970 at surface water temperatures between 19.0 and 27.0°C (Fig. 4). There was no evidence of breeding when temperatures rose above 27.0°C in January and early February, although GSIs remained high resulting in some breeding when temperatures dropped again. In most cases, a water flow was maintained through the ponds and on at least one occasion breeding occurred shortly after the water level in the pond was raised. Examination of GSIs indicated, that some fish did not breed, even though they were large enough. Ovaries generally obtained ova of two size ranges suggesting that multiple breeding occurs during one season. Sometimes a few large ripe ova were left behind in the ovary, suggesting that one spawning had already taken place, and large numbers of medium sized ova remained suggesting a second spawning would occur shortly.

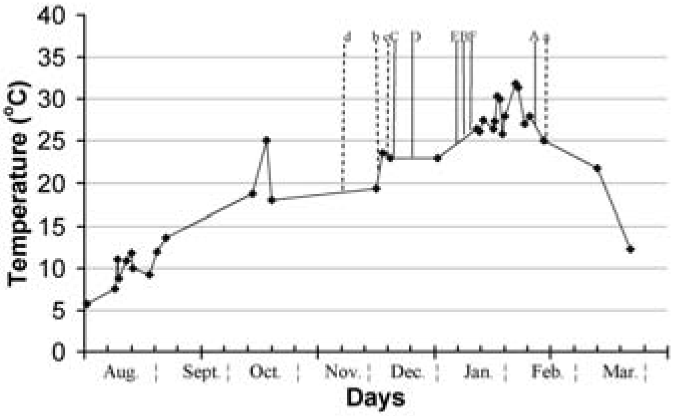


Figure 4. Pond surface temperatures from August 1967 to March 1968 showing times at which *A. agassizii* bred, (vertical solid lines and capital letters): **A**, 10.ii.68; **B**, early in -.i.68; **C**, 10.xii.67; **D**, 20.xii.67; **E**, 2.i.68; **F**, early in -.i.68;. Dotted lines and lower case show times of year they bred during other years, when temperatures would have been similar: **a**, 14.ii.69; **b**, 1-4.xii.70; **c**, 9.xii.70; **d**, mid -.xi.70.

A pond was stocked with 133 fish from Barren Box Swamp on 23.xi.67. The first larvae were found in this pond on 5.i.68. They were 11.0 and 12.0 mm in length, suggesting they were about 25 days old based on the size and growth of some other ambassid larvae (e.g. Ivantsoff *et al.* 1988). Ten days later on 15.i. 68 at a temperature of 21.7°C specimens from a shoal of larvae measured 4.1-4.5 mm (10 days old approx.) and from another shoal were 11.6-12.0 mm (25 days old approx.) suggesting that three spawnings had occurred over this period. It is estimated that they would have bred around the 10.xii.67, 20.xii.67 and 2.i.68 respectively when water temperatures were between 23 and 24.0°C for the first two spawnings and 25°C for the third (Fig. 4. C, D and E).

A second pond was progressively stocked with fish from 31.x.67, with a final stocking of 110 fish from Barren Box Swamp on 2.xii.67, making a total of 146. Males were releasing milt on 22.xi.67 but females were not yet ready to spawn. On 29.iii.68, 128 adults and 214 + young were recovered from the pond. The mean total length of young was 22.5 mm ± SD 3.56 (n=50) suggesting that spawning occurred in early to mid January when temperatures were around 26.°C (Fig. 4.F), based on the growth rate of *A. agrammus* (Semple 1985b).

In a third pond stocked with 259 fish on 10.iv.68, only 59 remained on 15.xi.68 when few males were running ripe, and a water flow through the pond had been maintained. On 14.ii.69 no breeding had occurred, but ova could be seen through the abdominal wall of fish. The water level was raised in the pond and additional planktonic food was added. On 16.x.69 males were running ripe, however many small fish were present (mean total length 28.1 mm ± 2.34, n=17, approx. 230 days old), indicating spawning had occurred late in the previous season, probably soon after adding food and raising the water level on 14.ii.69 when temperatures had dropped to about 26.0°C (Fig. 4a). On 29.xii.69 a male and female from the pond were easy to strip but fertilisation was not successful (surface and bottom water temperature were 25.6 and 24.9°C respectively). Fish were still ripe on 23.i.70 (surface temperature 27.2°C). No breeding had occurred by 3.iii.70 when surface temperature had dropped to 22.2°C. On 3.xii.70 30 adults remained in a pond and no breeding had occurred during the previous summer; on 9.xii.70 one egg and five larvae 2.6-4.4 mm (approx. 5 days old) were retrieved in a plankton net (surface temperature around 23.0°C) (Fig. 4. b,c), suggesting that egg development had been very quick, approximately 1 day. By 14.ii.71 the larva had reached 24.0-26.0 mm (approx. 75 days old) and by 25.v.71 all had been predated by cormorants. These adult fish were two years old at spawning, since no spawning occurred the previous year.

In a fourth pond, 262 four month old fish were stocked in April 1968. By 28.xi.68, 176 had survived (21.5 – 37.1 mm Mean 27.7 mm ± SD 3.31 (n= 46)). On 17.ii.69 young fish were sampled from the pond (10.0-25.0 mm Mean 17.8 mm ± SD 3.49 (n=20)) (surface temperature 31.7°C), indicating breeding had occurred a couple of months earlier at temperatures around 26.0°C (Fig. 4.B), and that parent fish had bred at one year old.

In a fifth pond, 32 and 4 fish from Barren Box Swamp were stocked on 17.i.68 and 14.ii.68 respectively. Males on the latter date were running ripe and water was continually run through the pond. On 1.iv.68 34 adults and 42 young were retrieved from the pond, the length of the young fish were 14.4 – 25.1 mm, mean 22.0 mm ± SD 2.51 (n=30) (approx. 50 days old, see Fig. 4. A). On 2.xi.70 seven adults remained and the pond was refilled. Two larvae 9.0 and 10.5 mm (approx. 30 days old see Fig. 4. d) were recovered from the pond on 15.xii.70 and a larva from this spawning had reached 33.0mm (145 days old) by 8.iv.70.

Embryonic development

The egg

Organogenesis in *A. agassizii* could not be followed in detail because only a single fertilised egg was collected which did not survive. The egg was, however, similar to most other teleosts, as described by Kuntz and Radcliffe (1915), Lagler (1956), Manner (1964) and many others, and the observations made were very similar to those recorded for other species of *Ambassis* (Semple 1985a and b, Nair 1957, Ivantsoff *et al.* 1988).

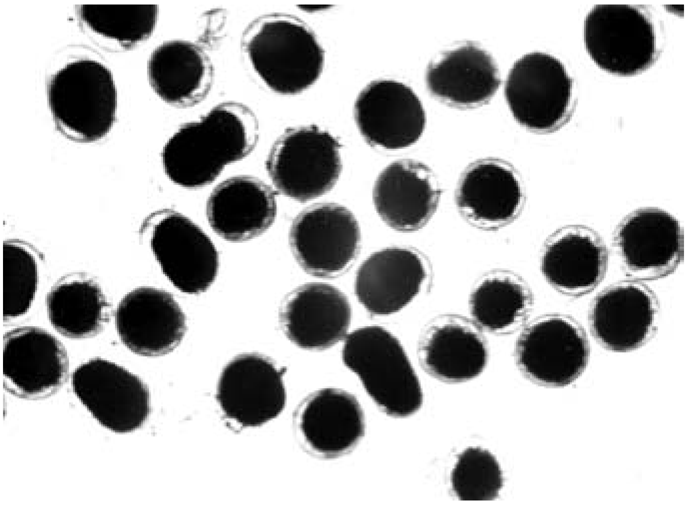


Figure 5. Stripped unfertilised ova of *A. agassizii* showing slight extension of chorion. These are on average 0.56 mm in diameter.

Three attempts were made to strip and fertilise ova from ripe females (on one occasion on 29.xii.69 the surface water temperature was 25.6°C) but on no occasion was fertilisation successful. Stripped, unfertilised eggs in which the chorion had extended and water hardened were 0.56 mm in diameter (Fig. 5). This, together with the size of large oocytes measured during counts (maximum diameter 0.61 mm), suggests that the mean diameter of water hardened eggs would be about 0.55 mm. When first stripped they had a layer of mucus around them which readily picked up detritus and eventually sloughed off.

The fertilised egg, measuring 0.61 mm in diameter, which was collected from a pond on 9.xii.70 at a surface water temperature of 23.0°C, did not develop any further when kept in a petri dish in the laboratory. It had a lot of debris attached to it, and was at cap stage at the onset of epiboly (Fig. 6). The egg was around 2 hours old based on the same stage in *Ambassis agrammus* (Semple 1985b). The egg was demersal, transparent, spherical and slightly adhesive. The perivitelline space was small, typical of a demersal egg. There was a large single oil globule present at this stage measuring 0.17 mm in diameter, and individual cells, numbering many thousands in the cap, could still be seen, unusual for this stage of development in most teleosts, but typical in ambassids. The yolk mass took up about 64% of the diameter of that enclosed by the extra-embryonic membrane. The yolk lay centrally within the chorion leaving a perivitelline space right around the yolk between the chorion and yolk.

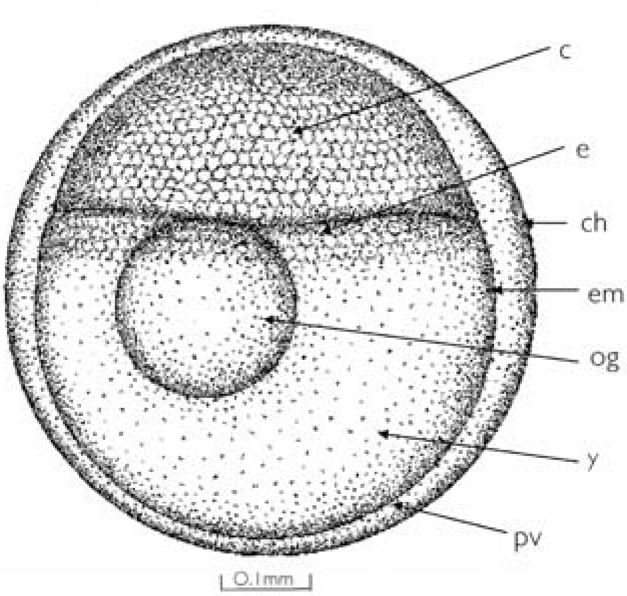


Figure 6. Egg of *A. agassizii* showing cap of cells at early epiboly. **c**, cap of cells; **ch**, chorion: **e**, epiboly; **em**, extra-embryonic membrane; **og**, oil globule; **pv**, perivitelline space; **y**, yolk.

The larvae

Since the mean egg diameter of *A. agassizii* was estimated at 0.55 mm, the larvae was estimated to be 1.6 mm at hatching, based on information from other ambassids where larvae reach about three times the egg diameter at hatching. Larvae between 2.7 and 15.0 mm in length were captured by dip net from ponds on eleven occasions when temperatures were above 20.0°C. On two of these occasions quite distinct size classes were caught (e.g. first pond discussed) suggesting that multiple breeding occurred in ponds at varying times and that young of different sizes (i.e. different ages Fig. 7) generally schooled separately. The smallest larva collected was 2.7 mm and was estimated to be 5 days old (cf. rate of growth in *Ambassis agrammus* Semple 1985b).

The terminology used in the subdivision of larval stages is based on Hubbs (1943), the pro-larva being the term used when the larva still possesses yolk. The small, 5 day old larva reported here was still a prolarva possessing a noticeable yolk sac (Fig. 8a), and the head region was quite large (0.68 mm in depth). The eyes were not pigmented, but a large optic capsule was present (0.3 mm in diameter). The mouth region was prominent but with no noticeable elements at this stage. Both the swim bladder (0.17 mm in length) and anal region were distinctly visible. Some small melanophores were present dorsally on the head, ventrally around the abdomen and along the musculature of the tail dorsally and ventrally. A prominent chromatophore was present in the region of the anus. The posterior distal end of the body musculature turned ventrally.

At 4.38 mm in length, approximately 10 days old (see Fig. 7 and Fig. 8b), the eyes were well pigmented and pigmentation was noticeable dorsally on the swim bladder and dorsally and ventrally along the vertebral column. Some elements of the mouth were now apparent, particularly the lower jaw, and a loop in the intestine had

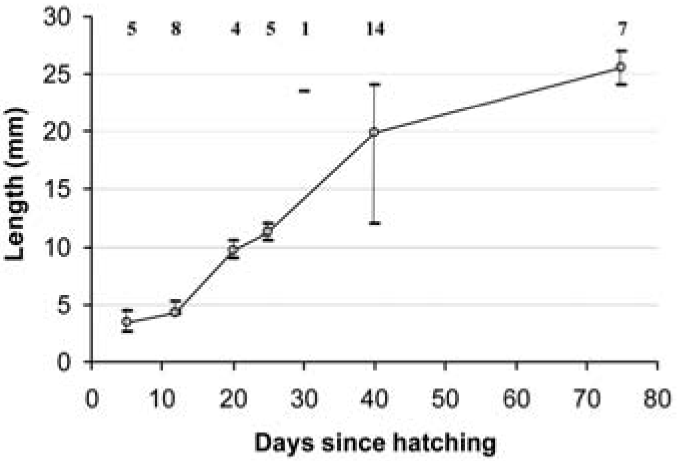


Figure 7. Growth rate of *A. agassizii* up to 75 days old based on early season and late season spawners. Bars indicate range of size at a given age. The late spawners would have had slower growth than early spawners particularly after the first month (30 days) because they would be going straight into autumn and winter. Numbers in sample are shown.

first appeared. Some fin rays in the dorsal, ventral and caudal fins started to appear, while the fin fold started to diminish in the dorsal and ventral caudal region. This stage appears to be an early post larva since yolk is not obvious and abdominal structures appear to be developing.

At 7.0 mm in length, approximately 20 days old (see Fig.7. and Fig. 8c), the abdominal region had shrunk, clearly showing the lack of yolk and the existence of intestine, and the mouth elements were visible. The bony structures of the vertebral column with the neural spines were now evident and the latter possessed pigment at their bases. The ventral neural spines were also pigmented at their distal ends. The dorsal, ventral and caudal fins were fully formed with their fin rays. The pectoral fins were present but not easily seen. The fin fold had now gone, and the swim bladder was triangular tapering posteriorly.

The changes in form that occur between 20 and 30 days mark the approximate period during which the post-larval stage terminates and the juvenile stage commences. Hubbs (1943) defines post-larva as “Larva following the time of absorption of yolk; applied only when the structure and form continues to be strikingly unlike that of the juvenile.”

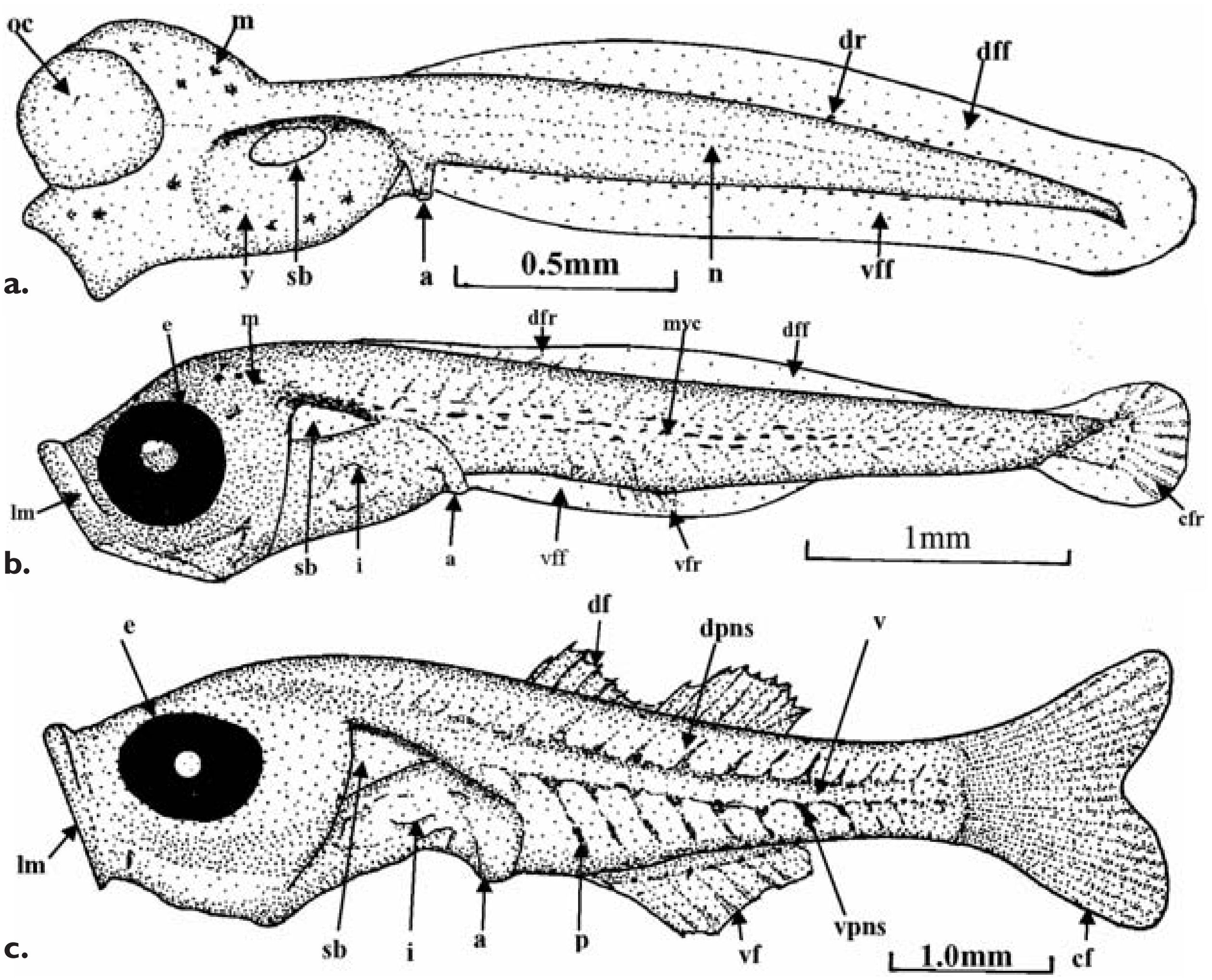


Figure 8. Larva of *A. agassizii* approximately **a)** 5 days old (2.70 mm in length). **b)** 10days old (4.38 mm) and **c)** 20days old (7.0 mm). **a**, anus: **cf**, caudal fin; **cfr**, caudal fin with rays; **df**, dorsal fin; **dff**, dorsl fin fold; **dfr**, dorsal fin rays just starting to form; **dpns**, dorsal pigmented neural spine, **dr**, dorsal row of pigment spots; **e**, pigmented eye; **i**, intestinal loops; **lm**, lower mandible; **m**, melanophores; **mvc**, melanophores along vertebral column; **n**, notochord first appearing; **oc**, optic capsule; **p**, pigment spots on ventral neural spines; **sb**, swim bladder with well pigmented dorsal region; **v**, vertebral column; **vf**, ventral fin; **vff**, ventral fin fold; **vfr**, ventral fin rays just starting to appear; **vpns**, ventral pigmented neural spines; **y**, yolk.

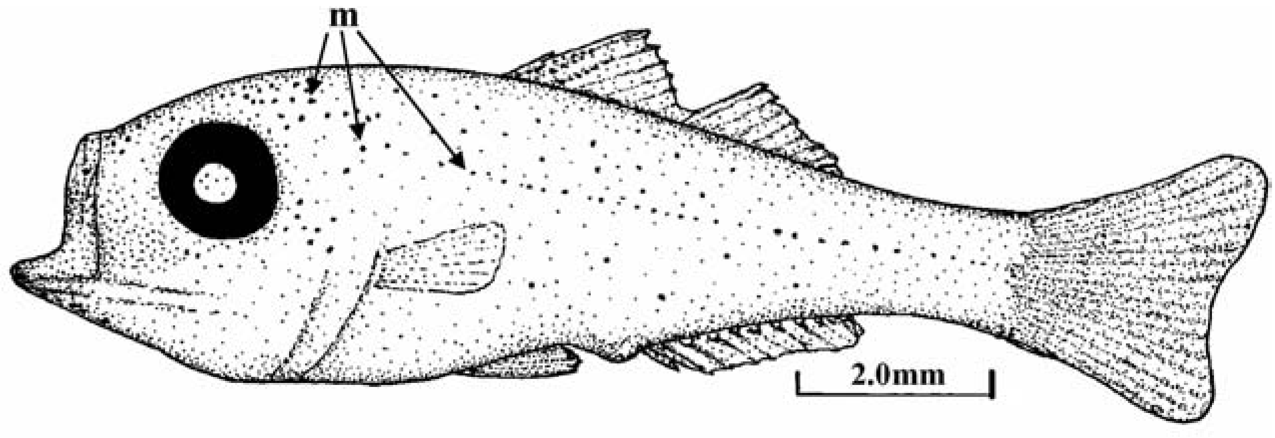


Figure 9. Juvenile (preserved) *A. agassizii* approximately 30 days old (13 mm total length). Note small melanophores distributed over body (m).

Juvenile fish

At 13.0 mm in length, approximately 30 days old (Fig. 7, 9) the scales start to appear and the larva starts to adopt the stream-lined appearance of a juvenile fish. Noticeable in the skin are small melanophores on top of the head particularly behind the eye, and mid laterally behind the eye on the operculum. Very sparse small melanophores also occur on the body with a distinct row along the mid lateral line, as well as along the base of the ventral fin, which extends ventrally towards the tail. The second and third long spines of the first dorsal fin also have a row of melanophores along them. In preserved specimens, the eyes remain pigmented black apart from the pupil, which remains paler, often white. All fins are now fully formed and all other features are similar to the adult. At 40 days, the fish are starting to experience declining water temperatures and growth rate appears to decline as winter approaches (Fig. 7).

Adults

The largest adults collected were from Willow Dam. A female 68.0 mm in total length (60 mm caudal fork length) and 3.88 g was caught on 17.ii.70, and a male 60.0 mm in total length (51.3 mm caudal fork length) and 2.51 g was caught on 12.xii.67. They had a GSI of 8.2 and 5.7 respectively and were approaching breeding condition. Males were generally smaller than females of the same age, and can be distinguished from each other when approaching breeding by the shape of the abdominal cavity and swim bladder. In females the dorsal outline of the abdominal cavity is curved (Fig. 1b and 3a), which makes the swim bladder sausage shaped. In males the dorsal outline of the abdomen is almost straight running towards the anus (Fig. 1a), resulting in the swim bladder becoming more bulbous at the posterior end and pointed at the anterior end. This is partly due to the swollen nature of gravid females. The salmon coloured lips around the anus in females become more tumid than in males as spawning approaches and both sexes have a small urinogenital papilla.

Adults generally have a glassy, golden to greyish hue dorsally, becoming paler ventrally along the tail region, but grading to a bright silvery white colour ventrally in the abdominal region, which is the lining to the abdominal cavity (see Fig. 1a and b). The scales on the dorsal region and ventral tail region have dark outlines making them clearly visible. A dark line of pigment exists mid-ventrally between the ventral and caudal fin, and the outer ring of the eye is silver. Sometimes, the back and caudal region is rather transparent, showing up some internal structures. Pigment spots are often present at the base of the dorsal and ventral fin rays. The pelvic and pectoral fins are almost colourless, apart from the whitish first filament of the pelvic fins. The eyes are a pronounced silver colour with a dark pupil in live specimens.

Gonad cycle

Mature ovaries, white to cream in colour, were divided at the anterior end for about a third of their length with one arm longer, larger and heavier in weight than the other (Fig. 3 b), particularly when ripe. They attained up to 18% of the body length with a GSI of 10.50 when fully ripe (Table 2).

Mature testes were divided into two arms for most of their length and each arm was more or less oval in shape longitudinally but flattened as both arms were compressed together. The two arms sometimes varied in length slightly but usually approached the same length, and they possessed a noticeable artery running up the outside of each arm. They were normally white in colour, reaching up to 17% of the body length and reached a GSI of 8.86 (Table 3)

Gono-somatic index

The number of gonad samples taken from fish from the wild, and from ponds at the research station to check on gono-somatic indices throughout the year, were limited and were taken on a random basis (Table 2 and Table 3). Quite a number of samples were from specimen collections at the Narrandera Fisheries Centre which had no locality or date data, but were included because of the limited material available. In some fish, gonads were too small or not found; GSIs obviously being very small.

Table 2. Biological data of *A. agassizii* females mainly from Willow Dam and ponds (Pond No.) at the Narrandera Fisheries Centre (NFC). TL, total length; CF, caudal fork length, SL. standard length. Not strip, means unable to extrude ova with gentle pressure to abdomen. Fish below 35 mm, and with a GSI less than 2 are considered to be non breeding.

Date	Location	Length in (mm) TL/CF	Fish weight in (gm)	Ovary weight in (gm)	Gono-somatic Index	Numbers of ova (fecundity) for ova diameter in mm. In brackets mean ova diameter, range in mm, and number measured (n).	Comments
23.i.70	Pond 2	43/38	1.054	0.1110	10.50	-	Ova granular. Not strip
8.i.70	Pond 2	45/39.2	1.2077	0.1130	9.39	TOTAL 2331 >0.19. 1391 > 0.44 (Mn 0.48, 0.44-0.61, n=12) 940 < 0.25 (Mn 0.22, 0.19-0.25, n =12)	Not strip.
17.ii.70	Willow Dam	39/36	0.6856	0.0131	1.91	-	Immature, 25.0°C
13.iii.70	Pond 2	47/41	1.1959	0.0652	5.45	TOTAL 728 >0.33 (Mn 0.42, 0.33-0.53, n=20) Fish dried up	Not spent. 22.2-20.9°C
13.iii.70	Pond 2	48/42	1.3538	0.0600	4.43	-	22.2-20.9°C
17.ii.70	Willow Dam	68/60	3.8851	0.3204	8.25	-	Ripe female. 25.0°C
17.ii.70	Willow Dam	56/49	2.1019	0.0689	3.28	-	Ripe. 25.0°C
17.ii.70	Willow Dam	32/28	0.3682	0.0043	1.17	Not possible to count. Few small ova nearly all broken down. Resorbing.	25.0°C
16.x.69	Pond 2	60/53	2.6904	0.2609	9.70	-	Spawned 8 months earlier
14.ii.69	Pond 2	56/50	2.0802	0.1586	7.62	Ovary dried. Small ova clumped and distorted. Not counted Large ova. 1319 (Mn. 0.33, 0.21-0.49, n=30).	24.4°C Ova through wall
23.xii.67	Pond 1	39.2/34.2	0.6730	0.0240	3.57	-	-
23.xii.67	Pond 1	46.2/39.9	1.0705	0.0312	2.91	-	-
23.xii.67	Pond 1	52.2/46.9	1.4836	0.0654	4.41	-	-
15.i.68	Pond 20	50.7/44.9	1.7052	0.0397	2.33	-	Spent
15.i.68	Pond 20	53.0/46.1	2.0582	0.0482	2.34	Ovary very small. Dried. Only about 20 recognisable ova. (Mn 0.28, 0.16-0.39, n = 7).	Spent. Ovary 3x1 mm
15.i.68	Pond 20	53.5/45.8	1.8625	0.0824	4.42	Ovary dried. A few small ova recognisable Not able to count.	Ovary 4.3 mm long. One arm 1.7 mm other 1.0 mm wide.
15.i.68	Pond 20	54.9/47.0	1.9300	0.0998	5.17	Small ova too small to count. Large ova. 1238 (Mn 0.29, 0.16-0.49, n=30)	Partly spent. Ovary 5 mm long.
15.i.68	Pond 20	55.1/48.1	2.2115	0.1269	5.74	Dried ovary. Not able to count small ova. Large ova - distal end. 1690 (Mn. 0.33, 0.23-0.43, n = 30).	Partly spent. Ovary 6 mm long 5 mm shortest arm. 5 mm wide.
15.i.68	Pond 20	55.6/47.7	2.3292	0.0522	2.24		Spent
15.i.68	Pond 20	56.4/49.0	2.3810	0.0835	3.51	TOTAL 992>0.12. (Mn 0.21, 0.12-0.30, n=20)	Partly spent
15.i.68	Pond 20	57.0/49.8	2.4118	0.1393	5.78	TOTAL 817> 0.33. (Mn 0.37, 0.33-0.45, n=20)	Not spent
12.xii.67	Willow Dam	41.4/35.8	0.7637	0.0422	5.53	TOTAL 2108 >0.21. 673>0.51 (Mn 0.55, 0.51-0.59, n=12) 1435< 0.34 (Mn 0.27, 0.21-0.34, n =12)	-
12.xii.67	Willow Dam	48.8/-	2.1047	0.0745	3.54	-	-
12.xii.67	Willow Dam	49.0/42.7	1.2595	0.0592	4.69	-	-
12.xii.67	Willow Dam	52.6/45.7	1.6271	0.0634	3.90	-	-

Date	Location	Length in (mm) TL/CF	Fish weight in (gm)	Ovary weight in (gm)	Gono-somatic Index	Numbers of ova (fecundity) for ova diameter in mm. In brackets mean ova diameter, range in mm, and number measured (n).	Comments
12.xii.67	Willow Dam	52.7/46.2	1.7195	0.0817	4.75	-	-
12.xii.67	Willow Dam	52.8/46.0	1.9268	0.0868	4.50	-	-
12.xii.67	Willow Dam	54.3/46.3	1.7721	0.0742	4.19	-	-
23.xi.67	Willow Dam	60/53	2.8030	0.2160	7.71	TOTAL 4547 >0.28 (Mn 0.39, 0.28-0.53, n=30). Small ova too difficult to count because of preservation	Ovary 7x8 mm, 20.6-22.8°C
23.xi.67	Willow Dam	62/54	3.2180	0.1250	3.88	TOTAL 5607 (Mn 0.21. 0.11-0.49, n= 50). Very few large ova.	-
23.xi.67	Willow Dam	67/59.5	3.6558	0.1860	5.09	-	-
23.xi.67	Willow Dam	59.0/51.0	2.1067	0.0789	3.75	Only ovary wall present with 98 medium to large ova attached. (Ova range 0.25-0.49).	Ovary 6.5x5.8 mm
23.xi.67	Willow Dam	59/52.5	2.4551	0.2437	9.93	TOTAL 5367 >0.11. 2002>0.38 (Mn 0.46, 0.38-0.55, n=20). 3365 < 0.23 (Mn 0.18, 0.11-0.23, n =20)	Ovary 11.0x10 mm Photographed
9.xii.69	Pond 2	59.0/51.0	3.2200	0.3339	10.37	TOTAL 3779 >0.19. 1840>0.38 (Mn 0.49, 0.38-0.57, n=13). 1939< 0.27 (Mn 0.22, 0.19-0.27, n =13)	23°C. Some ova stripped 0.56mm in diameter when water hardened.
14.?.67	Pond 2	53.0/47.0	1.1505	0.0216	1.88	Not counted. (Mn 0.06.0.04-0.08, n=20). Fish dried up.	Ovary 8x5 mm
-	NFC	56.0/50.0	1.0740	0.01920	1.79	All ova < 0.06, most ova < 0.04. Ova not counted.	Ovaries 6x3 mm
-	NFC	40.0/36.0	0.4347	0.0077	1.77	All ova < 0.10, most ova < 0.04. Ova not counted.	Ovaries 4x3 mm
-	NFC	S.L 47.0	-	-	-	All ova < 0.06. Fish partly decomposed.	Ovary 6x4 mm unripe
-	NFC	56.0/51.0	1.2988	0.0106	0.82	All ova < 0.06	Ovary 7x2 mm unripe
20.i.60	Bokhara R. Brewarrina / Enngonia	37.0/33.0	0.6404	0.0201	3.14	TOTAL 1248 > 0.08 626 > 0.25 (Mn 0.34, 0.25-0.42, n=30) 622 < 0.24 (Mn 0.14, 0.08-0.24, n-30)	Ripe
-	-	56.0/48.0	-	-	-	TOTAL 3755 >0.15. 1103>0.35 (Mn 0.40, 0.35-0.48, n=30). 2652< 0.26 (Mn 0.20, 0.15-0.26, n =30)	-
29.vi.67	NFC	54.0/46.0	1.1419	0.0229	2.01	Fish dried up. All ova < 0.06 too small to count	Ovary 6.0x2.5 mm
17.xi.67	Willow Dam	63.0/55.0	2.2333	0.1199	5.37	TOTAL 8999 > 0.08 1777 > 0.19 (Mn 0.26, 0.19-0.35, n=30) 7222 < 0.18 (Mn 0.12, 0.08-0.18. n=30)	-
1.ii.67	NFC	48.5/43.0	0.7033	0.0110	1.56	Fish dried up. All ova < 0.06. Not counted	Died after anaesthesia
1.ii.67	NFC	46.5/44.0	0.4551	0.0034	0.75	Fish dried up. All ova < 0.06. Not counted.	Died after anaesthesia
Pre 1964	Wentworth	50.0/46.0	1.0932	0.0195	1.78	All ova < 0.08	Ovary 5x3.5 mm
-	NFC	45.0/40.0	-	-	-	897 ova (very few ova and dried up)	-

Date	Location	Length in (mm) TL/CF	Fish weight in (gm)	Ovary weight in (gm)	Gono-somatic Index	Numbers of ova (fecundity) for ova diameter in mm. In brackets mean ova diameter, range in mm, and number measured (n).	Comments
-	NFC	53.5/48.0	-	-	-	Ova not able to count. Fish dried up.	-
-	NFC	61.0/55.0	2.1919	0.1668	7.61	TOTAL 6506 >0.15 2607 >0.29 (Mn 0.39, 0.29-0.48, n=30) 3899 < 0.29 (Mn 0.20, 0.15-0.29, n=30)	-
-	NFC	54.0/49.0	1.7636	0.0995	5.64	TOTAL 5326 > 0.12 2076 > 0.23 (Mn 0.33, 0.23-0.41, n = 30) 3250 < 0.21 (Mn 0.18, 0.12-0.21. n = 30)	-
-	NFC	39.0/35.0	0.5920	0.0225	3.80	TOTAL 1764 > 0.08 487 > 0.23 (Mn 0.31, 0.23-0.39, n = 30) 1277 < 0.21 (Mn 0.14, 0.08-0.21, n = 30)	-
-	NFC	58.5/53.0	2.4051	0.0511	2.12	TOTAL 9966 > 0.08 (Mn 0.13, 0.08-0.20, n = 30)	-
-	NFC	56.0/51.0	1.9253	0.0561	2.91	TOTAL 2281 > 0.10 694 > 0.31 (Mn 0.39, 0.31-0.53, n = 30) 1587 < 0.25 (Mn 0.16, 0.10-0.25, n = 30).	-
-	NFC	39.5/36.0	0.5273	0.0107	2.03	Most ova < 0.08. Very few up to 0.14mm	-
-	NFC	43.5/40.0	0.8845	0.0249	2.82	380 ova very indistinct mostly broken down (Mn 0.28, 0.12-0.58, n = 30)	-

The single female fish sampled in mid winter had a GSI of 2.01 (Table 2) supporting the view that gonads were small and inactive at this time. Twenty one percent of females at adult size (mostly > 45 mm) taken between mid October and early March, the breeding period, had a GSI below 3.00 suggesting that a proportion of the population does not breed each year which is supported by pond observations. The remaining females had variable but higher GSIs (max. 10.50 Table 2).

Close examination of these ovaries revealed oocytes at different stages of development, including a few unshed ova from a recent spawning, and much smaller ova developing for a future breeding. GSIs would drop significantly immediately after a spawning which is supported in part by the high range in monthly GSIs during the breeding season (Fig, 10). This, together with the presence in the ovary of groups of oocytes at different stages, provided evidence that they spawn a number of times during one season (multiple spawners). Some large oocytes were resorbing in mid to late February when GSI maxima (Fig. 10) were starting to decline. The breeding season extends from early November to February when maximum GSIs are above about 8 (Fig.10).

The lowest male GSI recorded was 0.63 and the maximum was 8.86 (Table 3). Clearly lower GSIs do occur because gonads were not found in some fish suggesting GSIs were very small and therefore were not determined. Only 2 male fish (6%) had a GSI of < 2.15 during the breeding season, middle of October to early March. This suggested that nearly all males that were old enough to breed did so. Fish caught during the breeding season had much higher GSIs, ranging from 3.0 to 8.0, and it was possible to obtain some milt from most of these by gentle pressure on the abdomen. The monthly GSI for males followed females closely and declined in February and March (Fig. 11), at the end of the breeding season. The numerous low GSIs in the undated fish suggest that these were taken during the non breeding season.

Fecundity

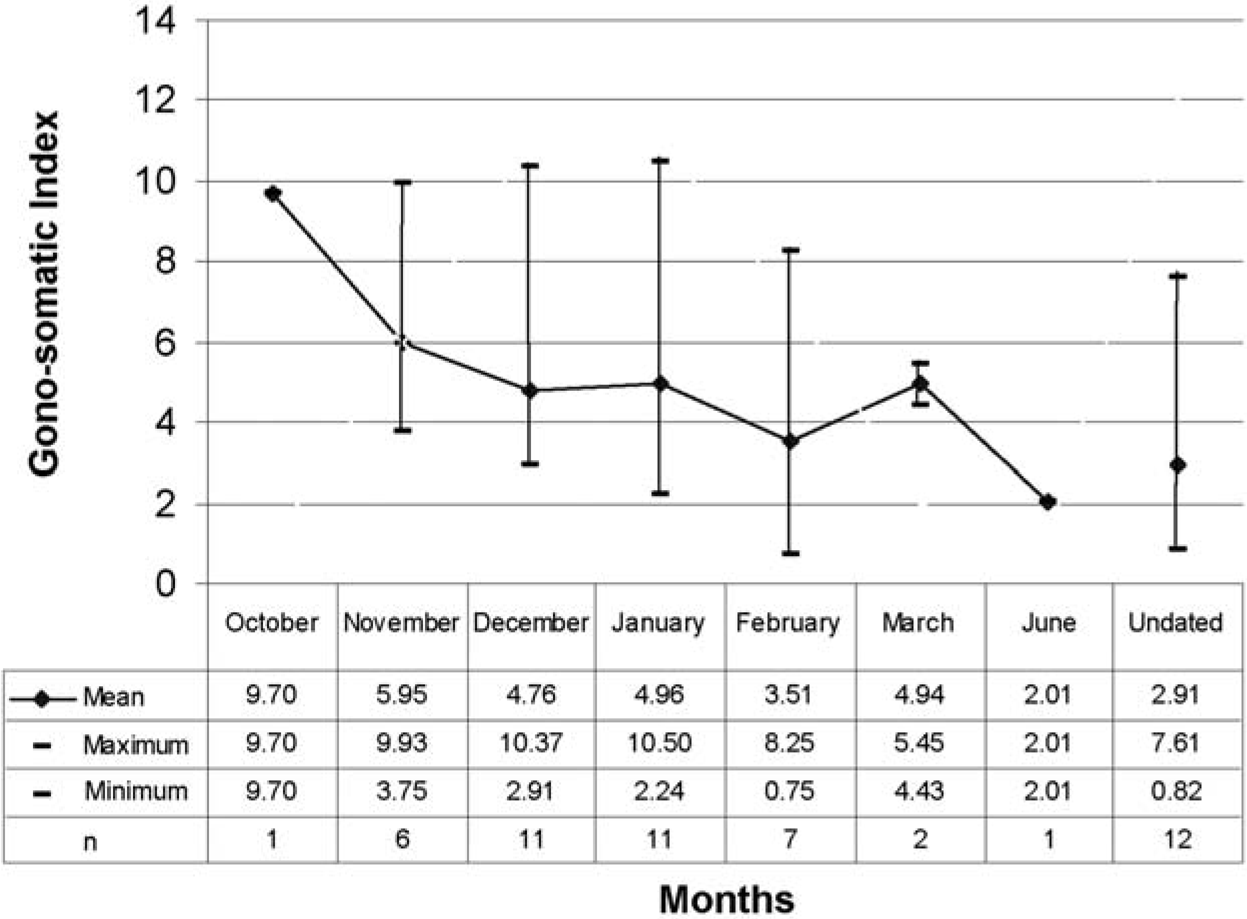
The measurement of fecundity, the number of eggs spawned annually, is difficult to determine in this species because it is a multiple spawner. Without information on the frequency of spawnings per fish annually, and the change in numbers of ova produced at subsequent spawnings as the season progresses, annual fecundity is difficult to determine. However, the numbers of ova in an ovary at any one time can be determined.

Egg counts from each ovary were in most cases divided into two size ranges which could be readily separated. They were not the same size in each gonad because it depended on how close each fish was to spawning. Only ova above about 0.08 mm were counted. The maximum count of 9966 was in an ovary possessing only small ova (0.08-0.20 mm, mean 0.13 mm). In ovaries containing

Table 3. Biological data of *A. agassizii* males mainly from Willow Dam and ponds at the Narrandera Fisheries Centre (NFC). **TL**, total length; **CF,** caudal fork length. Under Location "unknown" superscript numbers indicate fish were in same sample probably taken from same location.

Date	Location	Length in (mm) TL/CF	Fish weight in(gm)	Ovary weight in (gm)	Gono-somatic Index	Comments
22.i.70	Pond 2	42/37	0.9458	0.0461	4.87	Milt runs free 27.2-23.9°C
8.i.70	Pond 2	41/36	0.8527	0.0637	7.47	Slight milt, Testes large.
17.ii.70	Willow Dam	51/44	1.5244	0.0477	3.13	-
13.iii.70	Pond 2	47/40	1.0722	0.0389	3.63	22.2-20.8°C
15.xii.70	Willow Dam	47/53	1.6226	0.0183	1.13	25.5°C
15.xii.70	Willow Dam	50/43	1.3041	0.1155	8.86	Well developed. 25.5°C
17.xii.70	Willow Dam	-	1.2847	0.1018	7.92	25.5°C
17.ii.70	Willow Dam	57/51	2.1668	0.0465	2.15	303 collected
16.x.69	Pond 2	59/52	2.6443	0.2267	8.57	Milt freely
3.xii.69	Pond 2	39/34.5	0.7950	0.0440	5.53	19.3°C
3.xii.69	Pond 2	36/-	0.7084	0.0279	3.94	-
3.xii.69	Pond 2	36/-	0.7961	0.0414	5.20	-
3.xii.69	Pond 2	34/-	0.6940	0.0356	5.13	-
14.ii.69	Pond 2	58/51	2.3856	0.1303	5.46	24.4°C
23.xii.67	Pond 1	45.7/39.5	1.0621	0.0587	5.53	Some milt
23.xii.67	Pond 1	47.9/42.1	1.1520	0.0555	4.82	Some milt
23.xii.67	Pond 1	51.9/45.1	1.6597	0.0620	3.74	Milt
23.xii.67	Pond 1	58.5/52.1	2.1889	0.1043	4.76	Milt
15.i.68	Pond 20	51.0/43.5	1.6789	0.0763	4.54	-
15.i.68	Pond 20	52.6/45.1	1.8331	0.0645	3.52	-
15.i.68	Pond 20	53.5/46.1	2.0264	0.0668	3.30	Not spent
15.i.68	Pond 20	55.0/47.5	2.0965	0.1044	4.98	Cyst in testes wall
12.xii.67	Willow Dam	41.9/36.0	0.7478	0.0490	6.55	-
12.xii.67	Willow Dam	44.8/38.8	0.9406	0.0423	4.50	-
12.xii.67	Willow Dam	46.0/40.0	1.0987	0.0692	6.30	Parasitised
12.xii.67	Willow Dam	53.1/45.0	1.7239	0.0767	4.45	-
12.xii.67	Willow Dam	56.8/49.5	1.9681	0.1481	7.53	-
12.xii.67	Willow Dam	58.0/50.3	2.3092	0.0949	4.11	-
12.xii.67	Willow Dam	60.0/51.3	2.5056	0.1421	5.67	-
23.xi.67	Willow Dam	56/50	2.4760	0.1460	5..90	Testes lobed, elongated. 145 collected
-	NFC	53.0/48.0	1.3273	-	-	Testes 0.5x4mm very small
-	NFC	47.0/42.0	1.0215	0.0320	3.13	Testes 6x8mm
-	NFC	35.0/33.0	0.4471	0.0186	4.16	Testes 6x4mm
-	NFC	39.0/35.5	0.5952	0.0059	1.68	Testes 4x3mm
-	NFC	-	-	-	-	Testes 4.5x3mm
20.i.60	Bokhara Riv	36.0/32.0	0.5924	0.0270	4.56	-
-	NFC	51.0/46.0	1.2933	0.0488	3.77	-
-	NFC	35.0/32.0	0.3426	0.0038	1.11	Testes small
-	Wentworth	43.0/38.0	0.8033	0.0167	2.08	-
-	Wentworth	45.0/40.0	0.5619	0.0051	0.91	-
Pre 1964	Wentworth	39.0/36.0	0.4717	0.0039	0.83	Not ripe
-	NFC	50+/47.0	1.3350	0.0278	2.08	Testes ?
-	NFC	41.0/33.0	-	-	-	-
-	NFC	48.0/42.0	1.4599	0.0136	0.93	-
-	Unknown ^{1}	61.2/56.9	2.4136	0.0289	1.20	-
-	Unknown ^{1}	57+/53.0	2.2471	0.0400	1.78	-
-	Unknown ^{1}	57.0/51.0	1.9934	0.0684	3.43	-
-	Unknown ^{1}	59.0/54.0	1.8418	0.0460	2.50	-
-	Unknown ^{1}	60.0/54.0	1.8965	0.0476	2.51	-

Date	Location	Length in (mm) TL/CF	Fish weight in(gm)	Ovary weight in (gm)	Gono-somatic Index	Comments
-	Unknown [2]	43.0/39.0	0.7453	0.0106	1.42	Sample No. (202)
-	Unknown [2]	57.0/51.0	2.0004	0.0870	4.35	Sample No. (202)
-	Unknown [2]	46.0/42.0	0.9922	0.0155	1.56	Sample No. (202)
-	Unknown [2]	43.0/39.0	0.7973	0.0144	1.81	Sample No. (202)
-	Unknown [2]	43.0/39.0	0.7626	0.0184	2.41	Sample No. (202)
-	Unknown [2]	44.0/40.0	0.8852	0.0134	1.51	Sample No. (202)
-	Unknown [2]	44.5/39.0	0.9076	0.0138	1.52	Sample No. (202)
-	Unknown [2]	37.0/34.0	0.5758	0.0065	1.13	Sample No. (202). Photo.
-	Unknown [2]	51.5/47.5	1.7004	0.0724	4.26	Sample No. (202)
-	Unknown [2]	41.0/37.0	0.7342	0.0075	1.02	Sample No. (202)
-	Unknown [2]	37.5/34.0	0.5078	0.0032	0.63	Sample No. (202)
-	Unknown [2]	36.0/33.0	0.4883	0.0049	1.00	Sample No. (202)
-	Unknown [2]	37.0/34.0	0.4565	0.0060	1.31	Sample No. (202)



	October	November	December	January	February	March	June	Undated
◆ Mean	9.70	5.95	4.76	4.96	3.51	4.94	2.01	2.91
- Maximum	9.70	9.93	10.37	10.50	8.25	5.45	2.01	7.61
- Minimum	9.70	3.75	2.91	2.24	0.75	4.43	2.01	0.82
n	1	6	11	11	7	2	1	12

Figure 10. Mean, maximum and minimum gono-somatic indices of female *A. agassizii* mainly from Willow Dam and the Narrandera Fisheries Centre. Bars indicate range of GSI for each month. n = number of specimens.

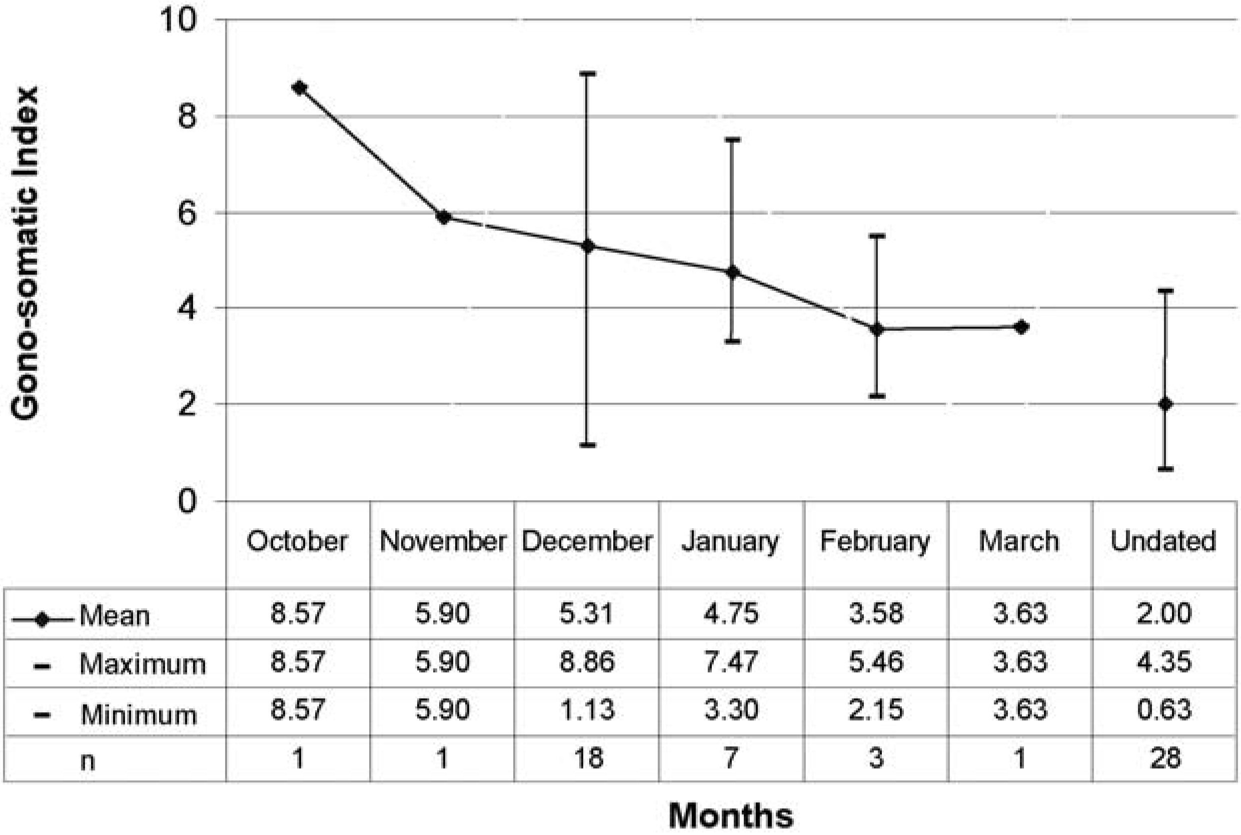
two distinct size ranges, the number of smaller ova varied between 622 and 7222 (mean 2563) and larger ova between 487 and 2607 (mean 1389). Total counts for ovaries varied between 380 and 9966 with a mean of 3257. There was an exponential trend for larger fish to have a larger total number of ova (Fig. 12).

Low numbers of nematodes were found in the ovaries of some adults, and a few cestodes were found in ovaries of some fish from Willow Dam

Growth and length frequency

Larvae and young fish in ponds grew to around 13 mm in 30 days, and about 25 mm in 80 days (Fig. 7). Larvae resulting from late summer spawning generally grew more slowly, because of the shorter growing period available due to the onset of colder winter months.

Monthly length frequencies were compiled for *A. agassizii* based on all fish measured and collected from Willow Dam and from specimens taken from ponds at



	October	November	December	January	February	March	Undated
—◆— Mean	8.57	5.90	5.31	4.75	3.58	3.63	2.00
- Maximum	8.57	5.90	8.86	7.47	5.46	3.63	4.35
- Minimum	8.57	5.90	1.13	3.30	2.15	3.63	0.63
n	1	1	18	7	3	1	28

Figure 11. Mean, maximum and minimum gono-somatic indices of male *A. agassizii* mainly from Willow Dam and the Narrandera Fisheries Centre. Bars indicate range of GSI for each month. n = number of specimens.

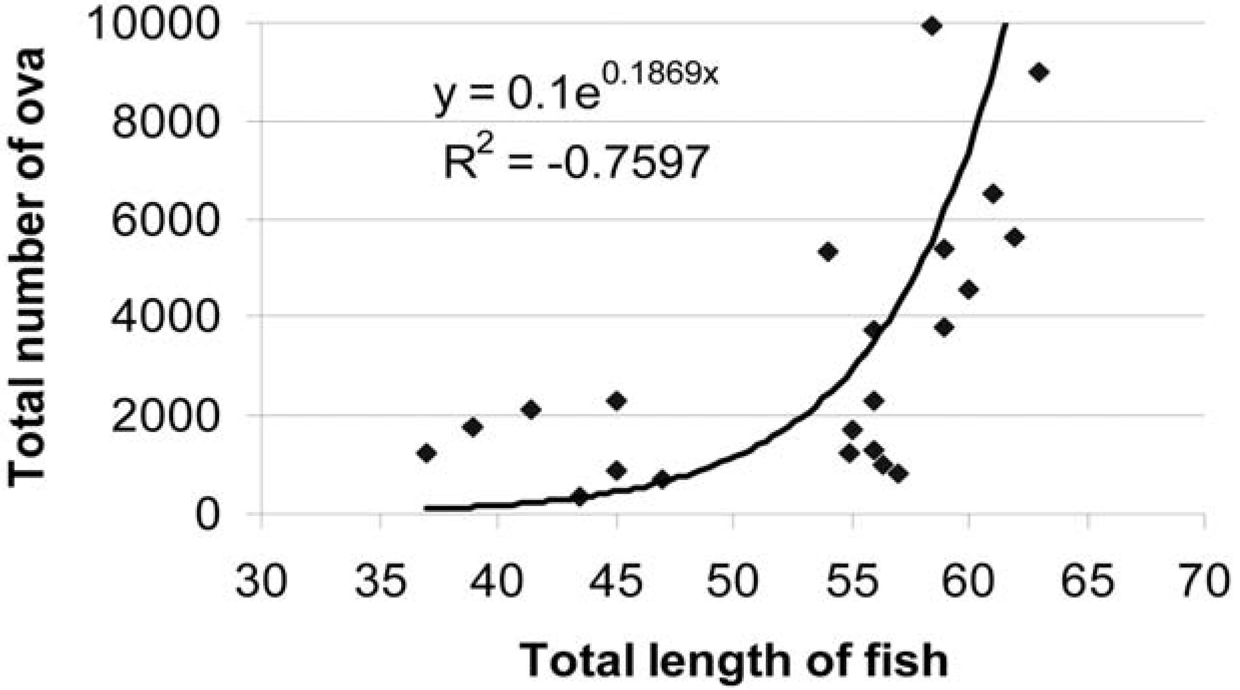


Figure 12. Exponential trend in fecundity of *A. agassizii* with total length of fish. Total numbers of ova were based on countable ova above 0.08mm in diameter.

the research station at known times of year (Fig. 13). Sufficient data were available only for the months of October through to April. This showed that recruitment of young to the population commenced in January and growth continued through to April. Although no data were available for winter months, the lengths of fish in October indicated that there had been little growth during this period. By December, these fish were large enough to breed. Some fish appear to survive to breed in a second season, a factor also supported by pond observations. These data support the timing of the breeding season.

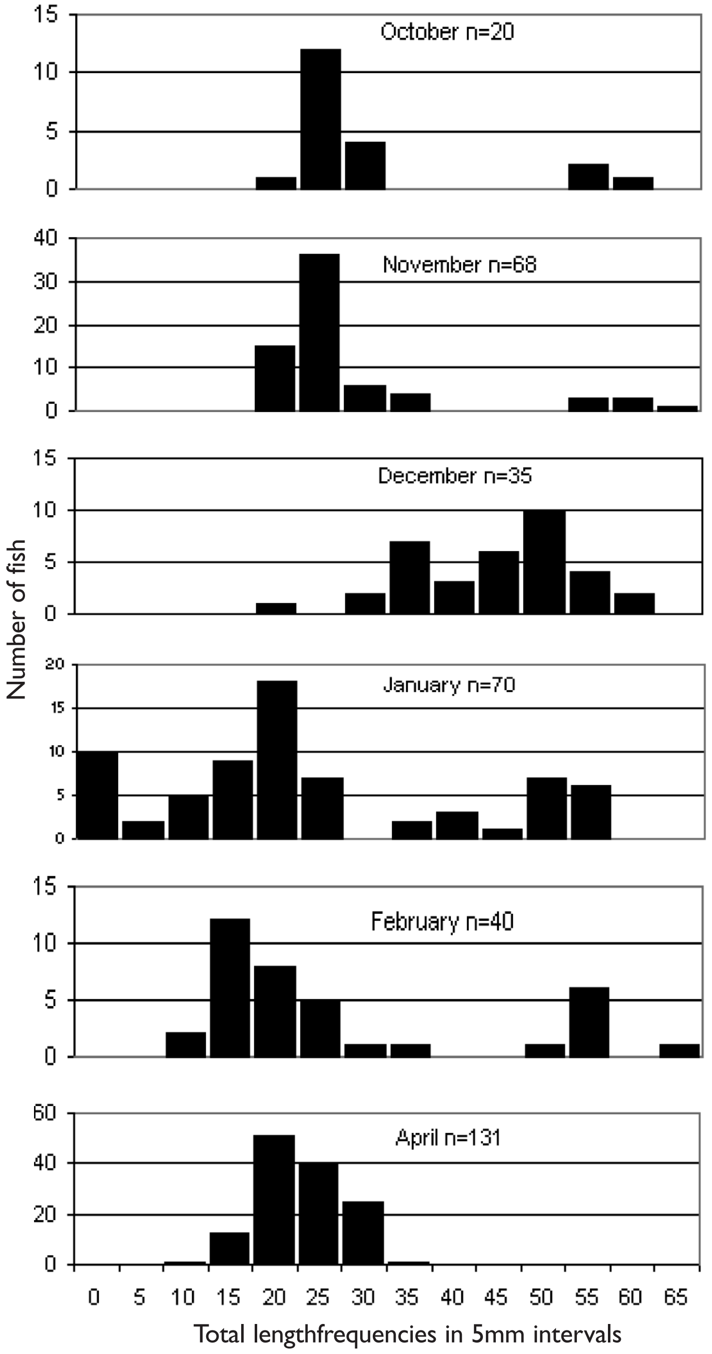


Figure 13. Monthly length frequencies of *A. agassizii* from October to April from ponds at the Narrandera Fisheries Centre and Willow Dam, showing new recruitment to the population in January.

Discussion

General

The brief comments on breeding biology of the Murray Darling *A. agassizii* published in Llewellyn (1980,1983) have been re-written by Allen (1996), and now have been attributed erroneously to this author (eg Morris *et al.* 2001). The new data provided here for *A agassizii* are compared with Llewellyn (1980).

Information from this study, supported by information from related species, has been used to piece together a picture of the breeding cycle. The results suggest *A. agassizii* is a multiple breeder, breeding during summer from November to early January when temperatures are rising between 19 and 27°C, and then cease when temperatures rise as high as 33°C, breeding again in February when temperatures drop again below 27.°C (Fig. 4). This extends considerably the earlier findings (Llewellyn 1980) in which breeding occurred at 23°C when the single egg was found. Fish bred at one year old and a few larger two year old fish also bred (see Fig. 13 and the 3rd pond stocking). Some females, large enough to be mature at one year old, did not produce eggs (see GSIs and 3rd pond stocking). Even though successful breeding did not occur in aquaria, increase in food supply such as appropriately sized planktonic crustaceans and small aquatic insects appeared to incite breeding activity. No assessment of their food supply in ponds was made, although fish growth in the wild is likely to be dependent on plankton blooms in spring and summer months, when aquatic insects are also abundant. Water flow and rising water levels in ponds appear to help in stimulating breeding but did not appear to be essential.

Comparison between the Murray Darling and Brisbane River (previously *A. nigripinnis*) populations of *A. agassizii*

Some aspects of the reproduction of *A. agassizii* from the Murray Darling population in this study, and from the Brisbane River coastal population (Milton and Arthington 1985) are compared (Table 4). The habitat and conditions in which the Murray Darling population lives is quite distinct from this coastal population. The former typically has spasmodic floods often in spring, with summer water temperatures occasionally reaching 34°C (Llewellyn 1975), while the Brisbane River has high flows in January to March outside their breeding period and where temperatures normally range from14 to 28°C (Milton and Arthington 1985). However Pusey *et al.* (2004) records temperatures as high as 33.6°C in northern coastal streams where this species occurs. The minimum temperatures where they occur in the Murray Darling (~ 10°C) seem to be similar to the coastal streams (11° C).

Most observations made in the Murray Darling population differed from the Brisbane River population examined by Milton and Arthington (1985). Catches of *A. agassizii* at Willow Dam (Fig. 2) suggest they school and move in response to running water over the weir, particularly during the early and late spawning period November and February, but also occasionally at other times (Fig. 2). Pusey *et al.* (2004) summarised observations on their movements and schooling behaviour in coastal areas, which often occurred in response to increased flows particularly in springtime. At Willow Dam some females were caught with high GSIs above 8.0 in mid November and mid February, and males above 7 in mid December, suggesting that spawning was imminent around these periods of reasonable flows. This activity was similar to that observed in ponds where flow through was maintained and breeding followed. In the Brisbane River population of *A agassizii* (Milton and Arthington 1985), the GSI rose to 9.5 in females and 7.5 in males during November and December, when river flows were extremely low.

Table 4. Comparison of the breeding biology of the Murray Darling and coastal population of *A. agassizii*, two north Australian species *A. agrammus* and *A. macleayi*, three Indian species *A. buruensis (=buroensis)*, *A. lala* and *A ranga*, and one brackish species *A gymnocephalus*. TL total length. *see other author.

SPECIES	*A. agassizii* (Inland) This study	*A. agassizii* (Milton and Arthington 1985 – coastal)	*A. agrammus* (Semple 1985b)	*A. macleayi* (Semple 1985a)	*A. buruensis* (Bates 1935)	*A. lala* (Sterba 1973a)	*C.(Parambassis) ranga* (Sterba 1973a and b)	*A. gymnocephlus* (Nair 1957)
BREEDING								
Time of year	Nov. Dec. early Jan. Feb.	Oct. –Nov. (spring)	Nov. Jan	All year round, peaks in early wet.	Oct (USA)	-	-	May. SW tip of India
Type	Multiple over breeding season	Egg scatterers in weed[i].	One to 4 consecutive days 1hr after dusk. Again in 2-6 weeks. Batches of 5 – 250 eggs laid at one spawning;	On 2-4 consecutive days at sunrise. Again in 2-6 weeks. Batches of 10-250 eggs laid	Every couple of weeks	During daylight Up to 20 times per day, over 4-5 days[d]. 100.s[i]	During daylight, up to 6 eggs at a time, up to total of 200	Spawn 7.00-8.00pm. Coastal on bar mouths
Temperature °C	19.0-27.0	Start at 22°C	27-35	27, (25-29[f])	21-27	Rising (27[g])	26-28°C[e]	-
Environmental condition	Flow. Occasional increasing water level	Rising temp. before summer floods	Late dry to early wet season in evening.	Early morning	Plenty of sunlight	-	Morning sunlight	Evening
In aquaria	No	Yes[f]	Yes	Yes	Yes	Yes	Yes	Yes but eggs collected
In ponds etc.	Yes	-	Yes	Yes and creeks	-	-	-	-
EGGS								
Diameter (mm)	0.61 (Mean 0.55)	Mean 0.6 mm	0.50-0.56	0.50-0.55	very small	-	-	0.6
No. of oil globules. Diam.	Single (0.17 mm)	-	Single (0.15-0.20 mm)	Single (0.15-2.0 mm)	-	-	-	Single (0.2mm)
Length of stage	(Approx. 24 hr)	5-7 days at 22°C (24-30 hr[f])	19-23 hr	21-23 hr at 25-28°C	12 hr	12-14 hr. 36 hr at 28°C	20-24 hr	17-18 hr.
Type	Demersal, slightly adhesive	Adhesive attached to aquatic plants	Demersal, adhesive generally on plants	Demersal, adhesive generally on plants	Randomly dispersed	adhesive among plants	adhesive among plants	Planktonic, sinking in still waters
LARVAE								
Length at hatch	<2.70 mm (Approx. 1.6 mm)	3.0 mm	SL 1.52-1.58 mm	SL 1.5-1.6 mm	very small	-	very small	1.12mm Floats at surface.
Eye pigmentation	None at hatching	-	3 days after hatching	None or lens at hatching	-	-	-	None at hatch 70 hrs, eye lens 21 hr.
Prolarva ended	Under 10 days	Free swimming 9 days (4-5 days[f])	3 days	3 days	Feeding difficult	Feed difficult 3 days[i]	3 days	4-5 days
End prolarva stage (mm)	-	-	SL 2.19-2.27 mm	2.5 mm[b]	-	-	-	-
ADULT								
Largest TL (mm)	68 mm	54 (3+) fem. 47male	70 mm[a]	100 mm (LCF)[a]	>38 mm.70mm[c]	>44 mm	70 mm	-

SPECIES	*A. agassizii* (Inland) This study	*A. agassizii* (Milton and Arthington 1985 – coastal)	*A. agrammus* (Semple 1985b)	*A. macleayi* (Semple 1985a)	*A. buruensis* (Bates 1935)	*A. lala* (Sterba 1973a)	*C.(Parambassis) ranga* (Sterba 1973a and b)	*A. gymnocephlus* (Nair 1957)
Largest weight (g)	3.88	-	-	-	-	-	-	-
Fecundity	380-9966. Mn 3257	230-740 >0.6 mm	312-2905	320-2360	-	-	200	-
Max. GSI fem	10.5	9.5	7[a]	3.5[a]	-	-	-	-
Max GSI mal	8.86	7.5	4.5[a]	1.5[a]	-	-	-	-
Finnage complete	7.0 mm. 20 days	-	SL 7.0mm 22-25 days	10 mm, 18 days[b]	-	-	-	-
Adults mature	TL ♂ 34 mm. ♀. 37mm 12months	♂ 32 mm[f]. ♀ 30 mm 12 months	LCF ♀ 26 mm, ♂ 27 mm[a] SL 30-40 mm. At 3 months	♂ 33 mm, ♀ 29 mm[a], 34 mm 3 months	38 mm (50 mm[h])	44 mm	-	-

*These authors are additional to key authors at head of table, indicated where appropriate. [a]Bishop *et al.* 2001; [b]Ivanstoff *et al.* 1988, [c]Sterba 1973a and b, [d]Breder and Rosen 1966, [e]Richter 1975, [f] Legget and Merrick 1987, [g]Innes 1966, [h]Axelrod and Schultz 1955, [i]Stoye 1935, [j]Legget 1984.

Ripening of gonads commenced in both populations in September reaching slightly higher GSIs in the Murray Darling population (Table 5), but winter GSIs dropped to a similar level of between 1-3 for females and 1-2 for males. Males in both populations invested a smaller proportion of their body weight for reproduction than did females. In the coastal population, breeding occurred in spring only at water temperatures of 22.0 -23.0°C in October and November, after several months of rising temperature. The Murray Darling population differed considerably from this.

Milton and Arthington (1985) indicated that egg development lasted 5-7 days in the Brisbane River, although Legget and Merrick (1987) stated 24-30 hr for the coastal populations in general. The Murray Darling population was similar to the latter estimate and to figures indicated for other ambassids (Table 4). Egg diameters of Murray Darling fish may be slightly smaller than Brisbane River fish. The coastal larvae at hatching (3.0 mm) were larger than the Murray Darling larvae estimated at 1.6 mm (< 2.7 mm minimum size found). The behaviour of larvae differed slightly in that the coastal larvae were dispersing amongst aquatic weeds and assuming a solitary life style at 8-12mm (about 40 days) while the inland larvae shoaled in different age groups at 10 days old (4.0 mm) and 25 days old (11.5 mm).

The fecundity of Brisbane River fish varied between 230 and 740 (Milton and Arthington 1985) based on oocytes larger than 0.6 mm and Pusey *et al.* (2004) reported 149 to 1574 ova for Mary River fish. In Murray Darling fish, all oocytes were ≤0.61mm, and there were two size groups generally present (> 0.08mm) in addition to many small oocytes (<0.02 mm), which were not counted. Fecundity was higher in Darling River fish (Table 2), and the various sizes of oocytes suggest that each spawning takes place over a couple of days or less, followed by at least a couple of weeks to allow for development of the next group of ova. This could take place a number of times during a season particularly if individual fish also breed in late summer. Annual fecundity is therefore difficult to determine. Some oocytes remaining in the ovary at the end of the breeding season were being resorbed. Milton and Arthington (1985) made no comment on the size distribution of oocytes or the possibility of multiple breeding in Brisbane River fish.

Although fish from the Murray Darling grew larger than from the Brisbane River (Table 4); fish from other coastal rivers have been reported to reach larger sizes (Legget 1984 reported 76 mm (*A. nigrippinis*) in coastal streams; Pusey *et al.* 2004 62 mm SL in SE Queensland streams). The largest Brisbane River fish (58 mm ♀) was 3+ years old (Milton and Arthington 1985), while the largest Murray Darling fish (60 mm ♂, 68 mm ♀) were estimated at 2 year old from length frequency data. In both populations, breeding commenced at 1 year old but Brisbane River fish matured at a smaller size than in Murray Darling population (Table 4).

Comparison of *A. agassizii* with other Ambassids

In Australia, of the six species in the genus *Ambassis* living in freshwaters (*A. agassizii, A. agrammus, A. elongatus, A. macleayi, A. miops* and A. sp. Northwest Glassfish) and the three species living in estuarine / freshwaters (*A. interrupta, A. marianus* and *A. vachellii*) (Allen *et al.* (2002), the breeding biology of only a few have been described. Some aspects of the reproduction of *A. macleayi*, and *A. agrammus* (Semple 1985a,b; Bishop *et al.* 2001; Ivanstoff *et al.* 1988 former species) have been described (Table 4.), and Coates (1990) provides information on *A. interrupta* from the Sepik River in Papua New Guinea. Merrick and Schmida (1984) state that *A. elongatus* has been bred in ponds and aquaria; and Larson and Martin (1990) state that the other Australian ambassids *Denariusa bandata* mature in their first year and breed in the early wet season; and *Parambassis gulliveri* produce ripe eggs and have juvenile fish in July.

Some overseas ambassids (*A. buruensis* (=*buroensis*) (Axelrod and Schultz 1955; Bates 1935); *A. gymnocephalus* (Nair 1957); *A. lala* (Innes 1966); and *Chanda (=Parambassis) ranga* (Sterba 1973a and b)) are included in Table 4 for comparison with *A. ambassis*.

Most ambassids are multiple spawners, spawning at one time or over a number of days and then again a number of weeks later; but often the time of day or night is important (see Table 4). In *A. agrammus* spawning occurs 1hr after dusk and in *A. macleayi* at sunrise (Semple 1985a and b), which can be an important isolating mechanism in sympatric species. Ambassids in general breed between 19 and 35°C on rising water temperatures. The Murray Darling population of *A. agassizii* appears to be the only one that breeds between 19 and 27°C and stops breeding when temperatures rise above 27°C and starts again when temperatures drop. Bishop *et al.* (2001) found that *A. agrammus* bred seasonally and *A. macleayi* had a uniformly low level of sexual development throughout the year with occasional small peaks. Some species respond to rising water levels or wet seasons, which seem inessential for *A agassizi,* and others seem very responsive to sunlight. An adequate food supply initiated by a set of suitable environmental conditions seems critical to successful breeding.

Egg diameters of Ambassids are very small, varying between 0.5 and 0.61mm, and generally have one large oil globule (see Table 4). The egg stages generally last from 12 to 24 hrs. Most species, like *A. agassizii,* have demersal adhesive eggs. Larvae of Ambassids are poorly developed when hatched, ranging between 1.5 and 3.0 mm in length**,** with no eye pigment and even no lens structure as in *A. agassizii*. Feeding starts at about 3 days in most species. Artificial feeding of larvae reared in aquaria is difficult, because live food of appropriate size and type is essential. Most ambassids mature at above 30 mm, but *A. agassizii* reaches this size at one year old, whereas *A. macleayi* can reach this size in 3 months (Semple 1985a). Based on size at which *A. agassizii* mature (34 mm ♂ and 37 mm ♀, Table 2 and 3), and rate of growth 25 mm at 75 days, it seems likely that most *A. agassizii* spawn at one year old, but larger fish up to 68 mm are spawning at 2 years old (Figure 13). Female A *agassizii* not breeding at 1 year old (approx. 20%) probably breed in the second year.

Fecundity of *A. agassizii* was slightly higher than other ambassids with a mean of 3257. However a single specimen of the estuarine *Ambassis marianus* 61.0 mm in total length from Narabeen Lake on 20.i.69 (10ppm) had 6642 ova (Mean diameter 0.35 mm, range 0.28-0.38mm) (Llewellyn unpublished data).

Comparison of egg and larval development of the inland population of *A. agassizii* with other NSW inland species

One of the main aims of this research was to assist with identification of eggs and larvae of Murray Darling Basin fish. A comparison of these eggs is presented in Table 5 (see authors in table). The eggs of *A. agassizii* can be differentiated from many other fish species by the single large oil globule in the yolk, the small diameter of the egg, the slight adhesiveness of the chorion just after spawning, and the time of year in which spawning is likely to take place (Table 5). The appearance of the cap stage, when individual cells are clearly visible, when many hundreds of cells are present, is characteristic of Ambassids. The only species overlapping with *A. agassizii* in egg diameter are *Leiopotherapon unicolor* (0.7 mm) which is generally slightly larger, and members of the *Hypseleotris* genus of which only the eggs of *H. klunzingeri* (0.48 mm) have been described. However *H. klunzingeri* normally has 2-5 oil globules which separate it from *A. agassizii*.

Because of the extended broken breeding season of *A agassizii,* annual fecundity is difficult to determine, but egg counts in the ovary of mature fish at any one time vary from 380-9955 ova. Ten of the inland fish species deposit in excess of 12,000 eggs up to many millions, and 16 species deposit less than 12,000 at each spawning (Table 5). Those producing lower numbers generally create nests, have specific spawning sites which are defended, have adhesive eggs or spawn amongst weeds where they have some protection. The multiple spawning of *A. agassizii,* and its likely dispersion of eggs amongst plants, would both be beneficial survival strategies.

Differentiation of larvae is generally more difficult because of the changes which occur as the larvae grow. Some important features at different stages of growth (e.g. Llewellyn 2005 Table 7) are: size at hatching and at commencement of post-larval stage; distribution of pigment including size and time of pigmentation of the eye; the shape of gut (May and Gasaway 1967) including the position of the anus; body shape; position and location of the swim bladder; and number and location of oil globules if present.

Ambassis agassizii can be easily distinguished at the early pro-larval stage by its length, position of the anus, lack of eye pigment when hatching, and number and location of melanophores. The anal position of *A. agassizii* prolarvae which is 3.3 (length from tip of head to anus divided by total length x10) is similar only to *Melanotaenia fluviatils*

Table 5. Eggs and spawning of fishes of the Murray Darling. aut = autumn, pk = peak.

Species	Common Name	Egg diam. (mm)	Oil globules	Colour and shape of eggs	Type of spawning	Time and temp. of spawning	Fecundity
Nematolosa erebi[p]	Bony bream	0.8 and above	-	Spherical	Pelagic / semi buoyant	20°C Spring early summer	100,000's
Oncorhynchus mykiss[w]	Rainbow Trout	3.0-5.0	?	Orange, opaque, spherical	Nest building non adhesive	Autumn/winter	200-12,000
Salmo trutta[w]	Brown Trout	4..0-5.0	?	Orange, opaque, spherical	Nest building, non adhesive	Autumn/winter	2,300-12,000
Retropinna semoni[u]	Australian Smelt	0.95	1	Pale amber, transparent spherical	Adhesive egg	15°C spring	200-3,000
Galaxias rostratus[q]	Murray Jollytail	1.4-1.7	1-12	Transparent, spherical	Random dispersal, non adhesive	10°C. Aug. Sep	2,330-7,000
Galaxias olidus[rx]	Mountain Galaxias	1.2-2.3	-	Spherical	Attach stones/scattered. Multiple	8-15°C Aug, Aut	Av.198, up to 434
Carassius auratus[w]	Goldfish	1.2-1.5	?	Greenish/yellow, transparent, spherical	Scatters over plants	Spring	300,000
Cyprinus carpio[w]	Common Carp	1.0	?	Creamy /yellow, spherical	Random dispersal, adhesive	17-26°C	16,000-2.2m
Tinca tinca[wa]	Tench	1.0	?	Yellowish, spherical	Adhesive, scatters on plants	22-25°C spring	275,000per/lb
Tandanus tandanus[h]	Freshwater Catfish	3.2	Small dispersed	Greenish/yellow, transparent, spherical	Nest building, non adhesive	>24°C	18,000-26,000
Gambusia holbrooki[tg]	Mosquito Fish	Viviparous	-	-	Viviparous. Multiple	Aug-Apr. Pk Oct	3-315 x9, mn 23
Melanotaenia fluviatilis[l]	Murray River Rainbowfish	1.03-1.15		Spherical, transparent	Tuft of adhesive filaments	19.9-22.5	100-150
Craterocephalus fluviatilis[o]	Murray Hardyhead	1.3	1-5	Transparent, spherical	Random dispersal, slightly adhesive. Multiple	24.7°C surface 23.6°C bottom	20-2,200
Craterocephalus stercusmuscarum[s]	Fly-specked Hardyhead	Up to 1.46	-	-	Adhesive filaments over egg. Multiple.	19-23°C. Oct-Feb.	5-126
Craterocephalus amniculus	Darling River Hardyhead	-	-	-	-	-	-
Ambassis agassizii	Agassiz's Glassfish	0.61 (0.55)	1	Spherical , transparent-	Multiple, slightly adhesive	Nov, Dec and Feb. 19-27°C	380-9,966
Macquaria australasica[f]	Macquarie Perch	4.0	numerous small	Pale yellow transparent	Random dispersal, adhesive	14-20°C	50,000-110,000
Macquaria ambigua[h]	Golden Perch	3.9	1 large, + small	Transparent, spherical	Random dispersal, pelagic	23°C	500,000
Maccullochella macquariensis[bc]	Trout Cod	2.5-3.6	1 large, + small	White opaque, spherical	Laid in batch, adhesive	14-22°C. Sept-Oct.	1,188-11,338
Maccullochella peelii[h]	Murray Cod	3,0-4.0	1 large + 20 small	Amber spherical	Adhesive, batch in log-	20.5°C	20,000-0.2m
Leiopotherapon unicolor[m]	Spangled Perch	0.7	1	Transparent. spherical	Random dispersal. Non adhesive	26°C surface	24,000-113,200
Bidyanus bidyanus[h]	Silver Perch	2.8	1	Transparent spherical	Random dispersal, pelagic	>23.3°C	500,000
Nannoperca australis[n]	Pygmy Perch	1.3	10-30	Transparent, spherical	Random dispersal, non adhesive	21°C	506-4,217
Perca fluviatilis[h]	Redfin	2.0	1	Transparent, spherical to polygon	Laid in batch, adhesive	>11.5°C	36,000-109,000

Species	Common Name	Egg diam. (mm)	Oil globules	Colour and shape of eggs	Type of spawning	Time and temp. of spawning	Fecundity
Gadopsis marmoratus[ed]	Freshwater Black fish	3.5-3.9	-	Yolky, orange	Demersal, adhesive, in logs	12-20°C	40-2,500
Gadopsis bispinosus[ev]	Two-spined Blackfish	3.5-4.0 ova	-	Yolky, orange	Demersal, adhesive, in logs	12-20°C Sep-Dec	157-350
Philypnodon grandiceps[j]	Flathead Gudgeon	1.8x0.8	Numerous	Transparent, Tear-drop shaped	Laid in batch, adhesive	18-28°C	up to 2,020
Philypnodon sp.	Dwarf Flathead Gudgeon	-	-	-	-	-	-
Mogurnda australis[k]	Purple-spotted Gudgeon	3.1x1.1	Many/dispersed	Transparent, elliptical	Laid in batch, adhesive	20-34°C	282-1,300
Hypseleotris klunzingeri[h]	Western Carp Gudgeon	0.48	2-5	Transparent, spherical	Random dispersal, adhesive	22,5°C Nov.	1,000-2,000
Hypseleotris sp1	Midgley's Carp Gudgeon	-	-	-	-	-	-
Hypseleotris sp2[i]	Lake's Carp Gudgeon	-	-	-	-	Oct-Dec	-
Hypseleotris sp3	Murray Darling Carp Gudgeon	-	-	-	-	-	-

[a] Breder and Rosen 1966 p.224, [b] Harris and Rowland 1996, [c] Ingram and Rimmer 1992, [d] Jackson 1978, [e] Jackson *et al* 1996, [f] Koehn and O'Connor 1990, [g] Krumholz 1948, [h] Lake 1967 a and b, [i] Larson and Hoese 1996, [j] Llewellyn (In press), [k] Llewellyn (2006) [l] Llewellyn (pers observations), [m] Llewellyn 1973, [n] Llewellyn 1974, [o] Llewellyn 1979, [p] Llewellyn 1983, [q] Llewellyn 2005, [r] O'Connor and Koehn 1991, [s] Milton and Arthington 1983a, [t] Milton and Arthington 1983b, [u] Milward 1965, [v] Sanger 1990, [w] Scott and Crossman 1973, [x] Marshall 1989.

and *Nannoperca australis* for those species shown in Llewellyn (2005 Table 7). In the later pro-larval and early post larval stages, key diagnostic features of *A. agassizii* are the large head, shape of lower jaw and mouth, large pigmented eye, and the distribution of melanophores.

The post larvae are more difficult to identify but shape, size and distribution of pigment are key characteristics.

Murray Darling distribution and status

The location of Willow Dam, the main collecting site in this study, lies close to the south eastern boundary of the distribution of *A. agassizii*. The other locations indicated in Table 1 lie within the general distribution as identified by Allen *et al.* (2002). The Northwest Glassfish (undescribed) (Allen *et al.* 2002) is the only other ambassid that approaches the range of the Murray Darling population of *A. ambassis*, which occurs in the adjacent Bulloo drainage.

Pond temperatures at the Narrandera Fisheries Centre occasionally reach extremes of 3.4 and 34.0°C with icing over of small ponds rarely occurring. River temperatures are generally less extreme than these, and it seems that the distribution of *A. agassizii* lies above the 10.0°C annual minimum river temperature (Mean over 15 years) (Llewellyn 1983). It seems likely that the extreme cold conditions at the Narrandera Fisheries Centre are just outside the natural range of this species and mortalities in ponds at times could have been due to these low temperatures.

The collection sites where *A. agassizii* were caught between 1960 and 1975 (Table 1) indicate a patchy distribution and difficulties in effectively sampling this species. Few *A. agassizii* were caught in this period despite the extensive fish survey (19, 900 specimens), which covered 189 sites west of the divide in all drainage basins (Llewellyn 1983) using a variety of techniques including electro-shocking. They were captured at only 2 sites, the Narran River and the Macintyre River during this survey. In addition, haul netting and other methods were carried out on hundreds of other occasions in dams, billabongs, lagoons and small creeks throughout the Murray Darling without success. Nevertheless, *A. agassizii* was caught on 17 occasions from 37 intensive sampling efforts at Willow Dam. Some other records during this period came from sampling by means of poisoning, and from gut analysis of cormorants. Sampling technique no doubt influences catchability.

Catchability and numbers of fish caught has been shown to vary with river conditions particularly floods (Llewellyn 1968) or wet seasons (Gehrke *et al.* 1995). Changes in catchability combined with short term sampling programs tend to over emphasise patchiness of records and infrequency of collecting *A. agassizii*, which makes it difficult to asses the true status of the population and consequently whether numbers have actually declined during the intervening 40 years. To further complicate this, it has been shown that other inland vertebrate populations are known to fluctuate dramatically in response to droughts and floods (wet periods) over many years (Bayliss 1987 for Kangaroo populations and Wong 1994 for waterfowl populations). Therefore it would seem logical that fish also fluctuate dramatically under the same environmental conditions.

The western Murray Darling population of *A. agassizii* is currently classified as an "Endangered Population" under the *Threatened Species Conservation Act 1995* (NSW Fisheries 2005a). This classification is applied because the population is considered to have been reduced to a critical level, or its habitat has been so drastically reduced that it is in immediate danger of extinction. Species in this category must be a listed vulnerable species, or be geographically isolated, near the limit of its natural range, and genetically distinct (NSW Fisheries 2005a). Current information supports the view that this population is geographically isolated from other populations.

In addition, *A. agassizii* is a member of the *Aquatic ecological communities* in the natural drainage system of the lower Murray River catchment, and the natural drainage system of the lowland catchment of the Darling River, both of which have been listed as "Endangered ecological communities – The ecological community (a group of different species living in a particular area) is likely to become extinct in NSW if threats continue" (NSW Fisheries 2005 b and c). However the Lachlan River, and Mirrool Creek drainage in which Willow Dam, the main collecting site for *A. agassizii* is situated, lie between the designated catchments of these endangered communities and therefore do not have such protection. The species is considered to be extinct in Victoria (Morris *et al.* 2001), although it was only ever recorded in the Mildura area in 1929 (Cadwallader and Backhouse 1983) and extinct in South Australia (Hammer and Walker 2004).

Morris *et al.* 2001 suggest it be categorised as Lower Risk (Least concern) in the IUCN red data list and in the ASFB (Australian Society of Fish Biology) list. They attribute declines in the southern half of the Murray Darling Basin to, "The removal of woody debris, associated with pressures of alien species, thermal pollution and the loss of natural flow regimes". It is interesting to note that both Redfin *Perca fluviatilis* and Mosquitofish *Gambusia holbrooki* were very abundant in most of the catches at Willow Dam, often adding up to many hundreds of fish.

Hard evidence does not exist to demonstrate that there has been a significant decline in *A. agassizii* populations over the past 40 years. Populations are obviously very small and patchy and because of this they need specific conservation attention. During the period from 1960-1975 they were not common, and most impacts listed above were clearly evident then and had been for some time. Problems of assessment of population size, and consequent categorisation under the TSC Act, are likely to be relevant to most inland fish species. In order to draw conclusions concerning the status of fish populations in the Murray Darling Basin, long term data are required to take account of cycles of wet and dry; and a better understanding of their biology and catchability is needed. Faragher and Rodgers (1997) indicated that electro-fishing was the most effective out of a number of fishing methods for NSW rivers, but still clarification was needed with respect to ability of any method to sample representatively from the full range of fish species and sizes present.

To achieve effective conservation of fish communities in the Murray Darling in the short term, it may be more appropriate to place them in categories stating that insufficient information is available on these populations, and channel limited funds into research to better understand these populations and the environmental requirements of inland fish species in general. It is likely that all species react in different ways to the same environmental conditions which they are all in tune with, and it is necessary to pay more attention to maintenance of conditions to benefit all species, than to earmark individual species for specific attention which may be favouring one species against another.

Conclusion

Ambassis agassizii was recorded patchily and unpredictably in the Murray Darling Basin in New South Wales north and west of Narrandera over the years 1960-1975. Its breeding biology is distinct from other species of inland fish, and the data provided here should assist in identifying their early development stages. Their breeding requirements will also help to formulate management strategies for inland waterways to ensure the survival of this and other species.

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References

Allen, G.R. 1996. Family Chandidae. Glassfishes, chanda perches. Pp.146-149 in *Freshwater fishes of south eastern Australia,* edited by R.M. McDowall. Reed Books, Chatswood, NSW.

Allen, G.R. and Burgess, W.E. 1990. A review of the glassfishes (Chandidae) of Australia and New Guinea. *Records of the Western Australia Museum.* Supplement 34:139-206.

Allen, G.R., Midgley, S.H. and Allen, M. 2002. *Field guide to the Freshwater Fishes of Australia.* Western Australian Museum, Perth.

Axelrod, H.R. and Schultz, L.P. 1955. *Handbook of tropical aquarium fishes. Family Centropomidae or Ambassidae*. New York McGraw-Hill. Pp. 573-575.

Bates, R.G. 1935. Breeding *Ambassis buroensis*. One of the Glassfishes. *The Aquarium*. Philadelphia Vol. **3**, no.**12** (April 1935): 278-280.

Bayliss, P. 1987. 8 Kangaroo dynamics. Pp. 119-134 in *Kangaroos, their Ecology and Management in the Sheep Rangelands of Australia,* edited by G. Caughley, N. Shepherd and J. Short. Cambridge University Press, London.

Belsare, D.K. 1962. Seasonal changes in the ovary of *Ophicephalus punctatus* Block. *Indian Journal of Fisheries* **9(1):**140-56.

Bishop, K.A., Allen, S.A., Pollard, D.A. and Cook, M.G. 2001. Ecological studies on the freshwater fishes of the Alligator Rivers Region, Northern Territory: Autecology. *Supervising Scientist Report* **145**; Supervising Scientist, Darwin.

Breder, C.M. and Rosen, D.E. 1966. *Modes of reproduction in fishes.* Natural History Press; New York. Pp. 396-397.

Cadwallader, P.L. and Backhouse, G.N. 1983. *A guide to the freshwater fishes of Victoria.* Government Printer: Melbourne. 249p.

Coates, D. 1990. Aspects of the biology of the perchlet *Ambassis interrupta* Bleeker (Pisces: Ambassidae) in the Sepik River, Papua New Guinea. *Australian Journal of Marine and Freshwater Research* **41**: 267-74.

Faragher, R. A. and Rodgers, M. 1997. Performance of sampling gear types in the NSW Rivers Survey. Pp. 251-268 in *Fish and rivers in stress – the NSW Rivers Survey,* edited by J.H. Harris and P.C. Gehrke. NSW Fisheries Office of Conservation and the Cooperative Research Centre for Freshwater Ecology: Cronulla and Canberra.

Gehrke, P.C., Brown, P., Schiller, C.B., Moffatt, D.B. and Bruce, A.M. 1995. River regulation and fish communities in the Murray-Darling River system, Australia. Pp. 363-375 in *Regulated Rivers: Research and Management.* **Vol 2**. John Wiley and Sons Ltd.

Hammer, M.P. and Walker, K.F. 2004. A catalogue of South Australian freshwater fishes, including new records, range extensions and translocations. *Transactions of the Royal Society of South Australia* **128**:85-97.

Harris, J.H. and Rowland, S.J. 1996. Family Percichthyidae. Australian freshwater cods and bassess. Pp. 150-63 in *Freshwater fishes of south eastern Australia,* edited by R.M. McDowall. Reed Books, Chatswood, NSW.

Hubbs, C.L. 1943. Terminology of early stages of fishes. *Copeia 1943* **(4)**:260.

Ingram, B.A. and Rimmer, M.A. 1992. Induced breeding and larval rearing of the endangered Australian freshwater fish trout cod *Maccullochella macquariensis* (Cuvier) (Percichthyidae). *Aquaculture and Fisheries Management* **24**: 7-17.

Innes, W.T. 1966. *Exotic Aquarium Fishes.* (18th edition). Philadelphia. Innes Publication Co. 541p.

Ivantsoff, W., Crowley, E., Howe, E. and Semple, G. 1988. Biology and early development of eight fish species from the Alligator Rivers region. *Technical Memorandum, Supervising Scientist for the Alligator Rivers Region.* Australian Government Publishing Service, Canberra 1988.

Jackson, P.D. 1978. Spawning and early development of the river blackfish *Gadopsis marmoratus* Richardson (Gadopsiformes: Gadopsidae) in the McKenzie River, Victoria. *Australian Journal of Marine and Freshwater Research* **29(3)**: 293-298.

Jackson, P.D., Koehn, J.D., Lintermans, M. and Sanger, A.C. 1996. Family Gadopsidae. Freshwater Blackfishes. Pp. 186-190 in *Freshwater fishes of south eastern Australia,* edited by R.M. McDowall. Reed Books, Chatswood, NSW.

Koehn, J.D. and O'Connor, W.G. 1990. *Biological information for management of native freshwater fish of Victoria.* Department of Conservation and Environment, Freshwater Fish Management Branch. Government Printer, Melbourne.

Krumholz, L.A. 1948. Reproduction in the western mosquitofish *Gambusia affinis affinis* (Baird and Girard) and its use in mosquito control. *Ecological Monographs* **18**:1-43.

Kuntz, A. and Radcliffe, L. 1915. Notes on the embryology and larval development of twelve teleostean fishes. *Bulletin of Bureau of Fisheries, Washington* **35:**89-134.

Lagler, K.F. 1956. *Freshwater Fishery Biology.* Brown, Dubuque.

Lake, J.S. 1967a. Rearing experiments with five species of Australian freshwater fishes, I. Inducement to spawning. *Australian Journal of Marine and Freshwater Research* **18**:137-53.

Lake, J.S. 1967b. Rearing experiments with five species of Australian freshwater fishes, II. Morphogenesis and ontogeny. *Australian Journal of Marine and Freshwater Research* **18**:155-73.

Larson, H.K. and Hoese, D.F. 1996. Family Gobiidae, subfamilies Eleotridinae and Butinae. Gudgeons. Pp. 200-228 in *Freshwater fishes of south eastern Australia,* edited by R.M. McDowall. Reed Books, Chatswood, NSW.

Larson, H.K. and Martin, K.C. 1990. *Freshwater Fishes of the Northern Territory.* Northern Territory Museum Handbook Series No. 1. Pp.37-42.

Legget, R. 1984. The Olive Perchlet *Ambassis nigripinnis, Fishes of Sahul* **1**: 29-30.

Legget , R. and Merrick, J. 1987. *Australian Native Fishes for Aquariums*. Griffin Press Ltd., Netley, SA.

Llewellyn, L.C. 1968. Tagging gives answers to fish growth queries. *The Fisherman. Journal of State Fisheries of New South Wales*. **Winter 1968**: 1-5.

Llewellyn, L.C. 1971. Breeding studies on the freshwater forage fish of the Murray-Darling river system. *The Fisherman, Journal of State Fisheries New South Wales.* **3**(13):1-12.

Llewellyn, L.C. 1973. Spawning, development and temperature tolerance of the spangled perch, *Madigania unicolor* (Gunther), from inland waters in Australia. *Australian Journal of Marine and Freshwater Research* **24**:73-94.

Llewellyn, L.C. 1974. Spawning, development and distribution of the Southern Pigmy Perch *Nannoperca australis australis* Gunther from inland waters in eastern Australia. *Australian Journal of Marine and Freshwater Research* **25**:121-49.

Llewellyn, L.C. 1975. *Temperature and River Height Data for Fifteen Inland Rivers of New South Wales 1963-1975.* (5 parts). New South Wales, State Fisheries: Sydney.

Llewellyn, L.C. 1979. Some observations of the spawning and development of the Mitchellian Freshwater Hardyead *Craterocephalus fluviatilis* McCulloch from inland waters of NSW. *Australian Zoologist* **20(2):**269-288.

Llewellyn, L.C. 1980. Family Ambassidae. Chanda Perches, etc. Pp. 140-141 in *Freshwater fishes of south eastern Australia,* edited by R.M. McDowall. Reed, Sydney.

Llewellyn, L.C. 1983. The distribution of fish in New South Wales. *Australian Society for Limnology. Special Publication*. **No. 7**.

Llewellyn, L.C. 2005. Breeding biology, and egg and larval development of *Galaxias rostratus* Klunzinger the Murray Jollytail from inland New South Wales. *Australian Zoologist* **33(2)**:141-165.

Llewellyn, L.C. 2006. Breeding and development of the endangered Purple-spotted Gudgeon *Mogurnda adspersa* population from the Murray Darling. *Australian Zoologist* (In press).

Llewellyn, L.C. in press. Spawning and development of the Flat-headed Gudgeon *Philypnodon grandiceps* (Krefft, 1864) (Teleostei:Eleotridae). *Australian Zoologist* **33(4)**:480-510.

Mackay, N.J. 1973. Histological changes in the ovaries of the Golden Perch (*Plectroplites ambiguus*) associated with the reproductive cycle. *Australian Journal of Marine and Freshwater Research* **24**:95-101.

Macleay, W. 1881. Descriptive catalogue of the Fishers of Australia. Vol. 1. *Pseudoambassis castelnaui*. *Proceedings of the Linnaean Society of New South Wales* **5(3)**:339.

Manner, H.W. 1964. *Elements of comparative vertebrate embryology*. The Macmillan Company: New York.

Marshal, J. 1989. *Galaxias olidus* in southern Queensland. *Fishes of Sahul* **5:** 223-225.

May, E.B. and Gasaway, C.R. 1967. A preliminary key to the identification of larval fishes of Oklahoma, with particular reference to Canton Reservoir including a selected bibliography. *Bulletin of Oklahoma Fisheries Research Laboratory.* No**. 5**, Contribution **(164)**: 1-42.

Merrick, J.R. and Schmida, G.E. 1984. *Australian freshwater fishes. Biology and Management.* Griffin Press Ltd. Netley, South Australia.

Miller, B. 1976. *Environmental control of gonadal cycles and egg laying periodicity in Little Pied and Little Black Cormorants in inland New South Wales*. Ph.D. Thesis. University of Sydney.

Milton, D.A. and Arthington, A.H. 1983a. Reproduction and growth of *Craterocephalus marjoriae* and *C. stercusmuscarum* (Pisces: Atherinidae) in south east Queensland, Australia. *Freshwater Biology* **13**: 589-597.

Milton, D.A. and Arthington, A.H. 1983b. Reproductive biology of *Gambusia affinis holbrooki* Baird and Girard, *Xiphophorus helleri* (Günther) and *X. maculatus* (Heckel) (Pisces: Poeciliidae) in Queensland, Australia. *Journal of Fish Biology* **23**:23-41.

Milton, D.A. and Arthington, A.H. 1985. Reproductive strategy and growth of the Australian Smelt *Retropinna semoni* (Weber) (Pisces: Retropinnidae) and the Olive Perchlet, *Ambassis nigripinnis* (De Vis), (Pisces Ambassidae), in Brisbane south-eastern Queensland. *Australian Journal of Marine and Freshwater Research* **36 (3)**: 329-341.

Milward, N. 1965. Development of the eggs and early larvae of the Australian smelt, *Retropinna semoni* (Weber). *Proceedings of the Linnean Society of N.S.W.* **90(2):**218-21.

Morris, S.A., Pollard, D.A., Gehrke, P.C. and Pogonoski, J.J. 2001. Threatened and potentially threatened freshwater fishes of coastal New South Wales and the Murray Darling Basin. *NSW Fisheries Final Report Series* **No. 33**. Pp. 53-55.

Nair, G.S. 1957. On the breeding habits and development of *Ambassis gymnocephalus* (Lac). *Bulletin of the Central Research Institute of University of Kerala India Series C. Natural Sciences* **5**:69-76.

NSW Fisheries, 2005a. What is currently listed? http://www.fisheries.nsw.gov.au/thr/thr-species/whats_listed.htm

NSW Fisheries, 2005b. *Fisheries Scientific Committee. Aquatic ecological community in the natural drainage of the lowland catchment of the Darling River*. New South Wales Fisheries. Ref. Nos. **FR 22.** File No. FSC 01/10.

NSW Fisheries, 2005c. *Fisheries Scientific Committee. Aquatic ecological community in the natural drainage of the lower Murray Catchment*. New South Wales Fisheries. Ref. No**. FR 16(2)**, File No. FSC 01/01.

O'Connor, W.G. and Koehn, J.D. 1991. Spawning of the Mountain Galaxias *Galaxias olidus* Gunther in Bruces Creek, Victoria. *Proceedings of the Royal Society of Victoria* **103 (2).** 1991: 113-123.

Pusey, B.J., Kennard, M.J. and Arthington, A.H. 2004. *Freshwater Fishes of North-eastern Australia.* CSIRO Publishing, Collingwood.

Richter, H. 1975. Spawning the Indian Glassfish, *Tropical Fish Hobbyist.* **24 (4).** Pp. 4-12 and 95-98.

Sanger, A.C. 1990. Aspects of the Life history of the two-spined blackfish *Gadopsis bispinosus* in King Parrot Creek, Victoria. *Proceedings of the Royal Society of Victoria* **102:** 89-96.

Scott, W.B. and Crossman, E.J. 1973. Freshwater Fishes of Canada. *Bulletin* **184**. *Fisheries Research Board of Canada, Ottawa.*

Semple, G. 1985a. *Ambassis macleayi*, - maintenance, reproduction and development of the Reticulated Perchlet. *Fishes of Sahul* **2(4):**90-94.

Semple, G.P. 1985b. Reproductive behaviour and development of the Glassperchlet, *Ambassis agrammus* Gunther (Pisces: Ambassidae), from the Alligator River system, Northern Territory. *Australian Journal of Marine and Freshwater Research* **36:**797-805.

Sterba, G. 1973a. *Dr. Sterba's Aquarium Handbook*. The Pet Library, Ltd.: London. Pp. 317-8.

Sterba, G. 1973b. *Freshwater Fishes of the World.* (Family Centropomidae Glassfishes, pp. 641-646). Vista:London.

Stoye, F.H. 1935. Gleanings. From German Aquarium Society in the 'Wochenshrift' with comments from the translator. *The Aquarium.* Philadelphia. **3**:107.

Steindachner, F. 1867. *A. agassizii* Chanda Perch. *Sitzungsberichte der Akademie der Wissenschaften, Wien* **lv (i):9.**

Wong, P. 1994. *The analysis of ten years of aerial survey data to determine the effects of hunting on game species of waterfowl*. Report. National Parks and Wildlife Service (NSW) Environmental Survey and Research Branch; and School of Mathematics and Statistics, University of Sydney.